The Journal of Animal Morphology and Physiology

VOLUME VII] DECEMBER 1960 [NUMBER 2

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The Journal of Animal Morphology and Physiology

This journal (*J. Anim. Morph. Physiol.*) published in two issues per year —one in June and the other in December, is the organ of the Society of Animal Morphologists and Physiologists and is issued free to all members of the Society.

Rates of Subscription

	India	U.S.A.	Other countries
Yearly for two issues......	Rs. 13.00	$3.00	£1-0-0
For one issue	Rs. 7.00	$1.50	£0-10-0

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STUDIES ON THE TIL LEAF ROLLER—*ANTIGASTRA CATALAUNALIS* (DUPONCHEL) (PYRALIDAE—LEPIDOPTERA)

1. External Anatomy of the Various Stages

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(8 plates)

Though many insects of the family Pyralidae (Lepidoptera) are pests of various crops and a good deal of information is available on their biology in India and abroad, the correct taxonomic position of some of these insects remains obscure. Kapur (1950) studied the morphological characters of some of the important genera of Crambinae and reassigned some species to their correct taxonomic positions, *e.g.*, *Chilo tumidicostalis* (Hampson), *Bissetia steniellus* (Hampson), *Chilotraea auricilia* (Dudgeon), etc. Among the Pyraustinae, because of the lack of detailed information on the morphological characters, there is still some confusion. Hence the external anatomy of one of the representatives, the common Til Leaf Roller—*Antigastra catalaunalis* (Dup.) including that of all its developmental stages, has been studied and is described in the present paper. These observations may also be helpful in the ready identification of all the different stages of the pest under field conditions, which is necessary in devising suitable control measures.

The eggs of the insect are laid singly on the underside of leaves or on flower buds. In 2-3 days they hatch and the larvae thus released generally pass through 5 instars before coming to the pupal state. The pupae after 4-5 days give rise to adults.

Egg (Plate 1, fig. 1)

About 0.36 mm. long and 0.25 mm. broad ; broadly oblong ; pale transparent when laid changing to yellow, then greyish and finally to red before hatching. Chorion transparent with the micropilar suture at the apex.

[J. Anim. Morph. Physiol., Vol. 7, No. 2, pp. 103-124, 1960]

1st Instar Larva (Plate I, fig. 2)

Body dirty white, head width 0.195 mm., total body length 1.68 mm. when freshly hatched, 2.48 mm. on the second day ; epicranial stem[1] very short, epicranial index[2] 6 ; adfrontal sclerite very narrow ; spinneret long, narrow, but prominent ; prothoracic shield very small, faint in the freshly hatched larvae, greyish on the second day.

2nd Instar Larva (Plate I, fig. 3)

Body greenish yellow ; head width about 0.31 mm. ; total body length 3.75 to 4.06 mm. ; epicranial stem 0.03 mm., epicranial index 5 ; adfrontal sclerite about 0.15 mm., adfrontal suture distinct ; spinneret long, narrow, as in the 1st instar larvae ; prothoracic shield darker than that of the 1st instar.

3rd Instar Larva (Plate I, fig. 4)

Body greenish yellow ; head width about 0.572 mm. ; total body length 6.5 to 7.5 mm. ; epicranial stem about ¼th the length of the frons ; adfrontal sclerites 0.15 mm. wide, adfrontal suture distinct ; mandibles wedge shaped ; apex of the teeth sharply pointed ; prothoracic shield dark brown ; pinaculi[3], one on each segment, brown.

4th Instar Larva (Plate I, fig. 5)

Body greenish with a prominent dark brown chalaza[4] on the subdorsal region of each segment ; structures same as in the 3rd instar, except in size ; head width nearly 0.68 mm. ; total body length 8.5-10.5 mm. ; epicranial index 3.2 ; width of the adfrontal sclerite 0.18 mm. ; pinaculi of the dorsal and subdorsal setae much chitinized ; prolegs slightly longer than that of the 3rd instar.

5th instar larva (Plate I, fig. 6)

Body greenish with three prominent dark brown chalaza on the dorsal and subdorsal regions of each segment excepting the three caudal ones ; total body length 14 to 16 mm. ; breadth across the head capsule about 1.02 mm. ; head light brown mottled with dark rectangular spots ; widest point at a level where the two adfrontals meet ; epicranial plates dorsoventrally wedge-shaped, dorsal area slightly broader than the ventral region ; first posterior seta Pl long, situated near the adfrontal suture ;

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[1] Median suture of the vertex.

[2] Ratio between the length of the frons and the epicranial stem—(Ripley, 1923).

[3] Small chitinized area surrounding the base of the papilla (Fracker, 1915).

[4] Raised chitinised area bearing the setae (Fracker, 1915).

second posterior seta P2 shorter than P1 and situated away from the adfrontal suture; posterior puncture Pa lying between P1 and P2 or rather slightly closer to P2; lateral seta L1 as long as P2 and situated near the lateral margin; lateral puncture La antero-mesad of L1; anterior seta A1 always longer than the second anterior seta A2 lying behind the former; third anterior seta A3 as long as A1, situated much behind and laterad of A2; anterior puncture Aa situated equidistant from both A2 and A3.

The microscopic setae and punctures in the vertical region arranged in a line with P1 and P2; first microscopic seta V1 equidistant from P2 and the second microscopic seta V2; V2, V3 and the puncture Va situated very close to each other, Va lying in between V2 and V3.

Frons (Pl. VII. fig. c) : Two third as long as the head capsule; frontal suture distinct, frontal seta F1 short, frontal puncture Fa situated mesad of the frontral seta; adfrontals irregular, first adfrontal seta Af1 shorter than F1, second adfrontal seta Af2 longer than and ventro-mesad of Af1, adfontral puncture Afa closer to Af2 than Af1.

Clypeus separated from the frons by the well defined clypeal suture and divided mesally into preclypeus and postclypeus; preclypeus concolourous with the frons or rather slightly darker, 2-3 times the median longitudinal width of the postclypeus; first clypeal seta C1 equidistant from F1 and the second clypeal seta C2.

Genal plate : Dark brown, separated from the hypopharynx complex by a well demarcated suture; genal seta G1 short; genal puncture Ga situated close to G1.

Ocelli (Pl. VII, fig. 6b) : Six in number, 5th ocellus remote from the ocellar ring and ventrad of ocellus 4, first ocellar seta O1 as long as the frontal seta and ventrad of the second and third ocellar setae O2 and O3; O3 laterad of O2; Ocellar puncture Oa situated between O1 and O2, but nearer to O2; Subocular setae three in number, first and second subocular setae So1 and So2 ventrad of ocellus 5 and the third subocular seta So3, ventrad of So2.

Antennae (Pl. VII, figs. e. f. g & h) : Four segmented, separated by coria to give free movements to joints; antecoria long; first and second antennal segments large, third smaller, fourth minute; first segment without any seta, second segment armed with 5 setae arranged in two rows, lateral seta or seta II_3 at the distal end longest, setae II_4 and II_5 conical; third segment trisetose, the two lateral setae longer than the central one; fourth segment unisetose with a single atenuate seta at the apex.

Mouth parts (Pl. VII)

Labrum (fig. c) : Dark brown, twice as long as broad, ventral area deeply cleft at the centre, with six setae and one puncture on each side; third labral seta Lb3 slightly longer than the rest; Lb4 situated laterad of Lb3 and much smaller; Lb1 and Lb2 medium sized, situated mesally with the puncture Lba; Lb5 diverted vantrad of Lb4 and Lb6.

Mandibles (fig. i) : 0.19 mm. broad and 0.22 mm. long, wedge-shaped, with a large scrobe bearing two usual setae; teeth six in number, outer and inner teeth blunt and slightly shorter than the rest; apex of the 2nd, 3rd, 4th and 5th sharp and bent forward; base of the mandible provided with a condyler knob with muscle attachment.

Hypopharyngeal complex (Pl. VII. fig. a) : Apparently appearing to be composed of two subgenae in the basal region and a congregation of basal lobes above it and the maxillary region on the two sides; cardo and stipes prominent; lacinia anastomosed with the first segment of the palpus; the galea at the distal end free, armed with two sensory cones; excepting the cardo, each of the maxillary lobes provided with a small seta at the tip; the enclosed space, in between the two maxillary regions, occupied by the submentum and mentum of the labium; the submentum divided into the upper raised ligula and a lower flat area with two small setae at its apex; mentum also divided into two regions—the proximal chitinized area bearing palpigers and the spinneret and a distal membranous area; labial palpi three segmented, the basal segment longest and thickest, armed with an apical papilla; second segment smallest, apical segment setaceous; spinneret provided with two sclerotized rings and a pair of minute setae on each side at the base; basal membrane wide, large and flexible allowing the spinneret to protrude and retract to and fro; tip of the spinneret broadly conical.

Thorax and abdomen (Pl. VIII) :

Total length 13-15mm.; breadth 4-5 mm.; general colour greenish-brown; pinaculi on all the segments on the dorsal and subdorsal regions dark brown; setae moderately long, no secondary seta; legs and problegs normal.

Prothorax (Pl. VIII, fig. a) :

Provided with a big transversely elongated chitinized shield at the subdorsal region on each side; setae I, Ia, II and IIa unisetose; subspiral setae IV and V unisatose, VI bisetose, VII trisetose and minute, VIII not distinct.

Mesothorax, metathorax and abdomen (Pl. VIII, fig. b, c, d & f) :

Two prominent pinaculi on each side of the dorsal and subdorsal regions; setae Ia, Ib, IIa and IIb long, III, IV and V short, unisetose; VI bisetose and VII trisetose; VIII distinct and unisetose: abdominal segment 1-7 with three dark brown pinaculi on the dorsal and subdorsal regions on each side; dorsal setae unisetose with a microscopic seta at the level of the spiracle; subspiral setae IV, V and VI unisetose, VII trisetose, VIII unisetose; segments same as the previous ones with an exception of unisetose seta VII; number of setae much reduced on segment 9 (fig. d), setae I and II unisetose; III not distinct; IV and V grouped together, unisetose; VI not distinct; VII unisetose; anal shield (fig. g) provided with 8 setae, arranged in three rows, first row consists of four setae, second and third row with two setae each; caudal legs similar to those of the prolegs.

Thoracic legs & abdominal prolegs (Pl. VII, figs. e & f)

1st to 3rd segments armed with a number of setae; coxites provided with five short setae at the base; basal segment of the legs proper armed with two setae near the inner margin towards the tip; second segment with six setae, all towards the tip; third segment with two minute setae at the inner margin of the tip; last segment terminated with a claw-like hook; basal part of the prolegs (fig. f) same as the thoracic legs, tips armed with bicrdinal crochets arranged in penellipse.

Pupa (Pl. VI, figs. e & f)

Total length 9-10 mm.; breadth 3-3.5 mm.; head capsule smooth, vertex small, semicircular, lying over the distinct epicranial stem; gena not visible; clypeus narrow, slightly extended on each side; antennae long, reaching upto the caudal end of the sixth abdominal segment, beyond the tip of the forewing; maxilla long, maxillary palpi prominent situated along the cephalic margin of the pro-and meso-thoracic legs.

Thoracic segments distinct; prothorax dorsally semicircular, prothoracic legs do not reach the second abdominal segment; mesothorax much bigger with the caudal region produced to a conical lobe; mesothoracic legs reach up to the distal margin of the seventh abdominal segment, half way between the tips of the antennae and the metathoracic legs; metathorax narrow, cephalic end highly concave, enclosing the caudal end of the mesothorax, distal margin flat; legs reaching upto the eighth abdominal segment; wing pads very prominent at the dorso-lateral region; forewing extended upto the sixth abdominal segment; hindwing, excepting a small area on the dorsal region mostly covered up by the forewing; wing veins few (Pl. IV, fig. c).

Abdominal segments 2-7 prominent, provided with two pairs of small setae, one on the subdorsal and the other on a line just above the elongate, oval, and easily distinguishable spiracle; seventh segment half as long as the sixth in both the sexes; segment 8 in the female much smaller than that of the male; genital opening of the male situated on the ventro-meson of the ninth segment and provided with a single slit-like opening flanked by two slightly elevated tubercles at the sides; the female aperture consisted of two slits, one each on the ventro-meson of the segments eight and nine; tenth segment produced to a long slender cremaster with tips armed with 4-6 short hairs.

Adult Moth

Head capsule (Pl. II, figs. a & b): head capsule simple, globular and flat dorso-ventrally; anterior region covered by broad, somewhat slightly convex frons demarcated laterally by the ocular suture extended from the antennal socket to the subgenal suture; frontal suture arch-shaped running across the head in between the bases of the two antennal sockets; gena long, narrow, demarcated ventrally by a genal suture; subgenal area narrow, conical, separated from the other cranial areas by a line running posterior to the posterior tentorial pits to the anterior articulation of the vestigial mandibles; compound eyes subglobular and approximated ventrally to some extent, projecting beyond the ventral region of the head capsule, inner margin slightly curved, with margin surrounded by a distinct ocular suture; two lateral ocelli (oc) situated on the dorsal part of the head just above the antenal socket, very prominent; third ocellus absent; dorsal region of the head capsule occupied by the vertex, occiput and the occipital areas; anterior tip of the vertex convex; coronal suture indistinct; occipital and the postoccipital areas practically the same as found in all other generalised insects; occipital area narrow, surrounded by the occipital and postoccipital sutures; postocciput situated posterior to it; postoccipital region all round the foramen magnum and the occipital arch incomplete ring-shaped, dorsoventrally widened into a semicircular lobe with an outward projection over the occipital foramen; laterally the postocciput thickened at two points to serve as occipital condyle; posterior region of the head capsule occupied largely by the occipital foramen with a bridge formation by the posterior arms of the tentorium and the hypostomal bridge at the ventral margin; anterior tentorial arms arising from the anterior tentorial pits extended anteriorly to the sides of the clypeus and then diverted backward through the anterior part of the head capsule to meet the posterior tentorial arms leaving the posterior tentorial bent, slightly inward, to form the tentorial ridge across the foramen magnum.

Antennae (Pl. II, figs. c, d, e & f) : Dark brown, filiform in both the sexes; consisted of 55-60 segments in the males, 60-65 segments in the female; proximal end of the scape (sc) concave and fixed up in the antennal sockets; rims of the sockets developed to knob like structures, the antennefers, articulated to the concavity of the scapes giving ball and socket joints; pedicel somewhat bulbous, slightly shorter than the rest of the segments, distal end concave, receiving concavity of the following segment; flagellum, excepting the tip, similar with convexity in the proximal end and concavity in the distal end, provided with two rows of long setae on the dorsolateral margin and a sensory cone at the ventro lateral side of the distal end; tip of the antennae slightly longer than the rest of the segments and armed with sensory cones numbering one in the females and more than two in the males.

Mouth parts (Pl. II, figs. a, b, g & h) : Labrum narrow, situated on the ventral side of the clypeus and produced ventrally into a mesal and two lateral lobes; filifers prominent, bristle like; epipharynx small but distinct; mandibles vestigial, represented by small triangular areas behind the filifers; maxilla most prominent, forming the slender proboscis, the galea (g), and the three basal segments, cardo, stipes and the lacinia; stipes curved mesally and articulated proximally with the triangular cardo; lacinia subcylindrical, small, attached to the stipes ventro-laterally; maxillary palpi (g) prominent, four segmented and hairy; labium not well defined, probably limited to two small rounded areas around the base of each labial palpus and short knob-like process in the mesal region; labial palpi (h) three segmented, basal segment well developed and curved forward and upward, second segment largest and stout, third segment narrow, smallest with a ventral sensory pit in both the sexes.

Thorax and thoracic appendages :

Like most other insects cervix or the intersegmental region between the head and the thorax almost membranous, sclerotization limited to a pair of 'Y' shaped sclerites, one on each side; dorsal arm of the 'Y' articulated with the episternum, the ventral arm remaining free; apex of the 'Y' attached to the occipital condyle.

Thorax (Pl. III):

Prothorax (fig. d) : Much reduced, but provided with two hairy outgrowths, the patagia and the parapatagia; the latter comparatively smaller and partly covered by the bigger and outer patagia; in the pleural region the pleural suture distinct giving rise to a slender pleural arm attached to the furcal arms; both the episternum and the epimeron distinctly separated; episternum much bigger and connected with the sternum by a small triangular sclerite, the precoxale, fused in front of the coxal cavity; presternum narrow and partly fused with the episternum horizontally; epimeron narrow, fixed up in the posterior concavity of the former; basisternum reduced to a very narrow region representing the edges of a deep median fold of the transverse internal ridge or sternacosta and separated from the sternallum by a well defined sternacostal suture, right up to the sternal pits; spinasternum narrow, represented by a vertical median strap-like process into two lateral arms posteriorly.

Mesothorax (figs. a & b): Prescutum small, separated from the scutum by a distinct suture, but directed ventrad to be almost invisible from the dorsal aspect; anterior margin depressed and produced laterally into two lateral prealars; the scutum very prominent, with anterior margin cleft; lateral margin caudad of the prealar, provided with tegular incision; the margin beyond the incision much flattened to form the triangular suralare or the anterior pivotal point of the wing; the structure posterior to the suralare bilobed; the anterior lobe or the adanale, articulated with the posterior margin of the wing; the posterior lobe or the postadanale suspended free in the muscles; the anterior margin of the adanale, the adnotale, produced further anterior close to the hook of the suralare giving additional support to the wing.

Tegula large, hairy and produced marginally by means of the tegular arms below and backward to pass beneath the base of the forewing; posterior margin elongated, reaching upto the base of the scutellum.

Scutellum more or less pyramid-shaped, situated posterior to the scutum ; anterior side bound with reversed 'V' shaped endotergal ridge dividing the mesonotum into scutum and scutellum ; posterior side demarcated by the intersegmental fold of the antecostal ridge between the scutellum and the metathoracic scutum ; post scutellum bearing a big phragma beyond the antecostal ridge developed to a great length, reaching upto the caudal end of the thoracic region between the metanotal plates.

The pleuron divided by a pleural suture into episternum and epimeron ; the episternum further divided by a transverse anepisternal suture into the dorsal anepisternum and the ventral katepisternum ; the katepisternum continued as a narrow precoxale but ultimately fused up with the furcasternum ; anterior to the katepisternum, the ventrolateral margin of the thorax, completely surrounded by a sternal lobe ; the epimeron demarcated into a somewhat subcircular region, the preepimeron of Shepard (1930) ; anterior margin of the preepimeron overlaped over the pleural suture and extended forward into the anepisternum ; subalare situated in the membrane over the epimeron with another small plate representing the second subalare behind it ; the mesothoracic spiracle situated partially concealed in the intersegmental membrane of the pleural region in between the pro-and meso-throax.

The basisternum broad, prominent with a ventral keel-shaped ridge ; furcasternum small, triangular ; median furcal arm fused with the posterior portion of the epimeron to form a brace across the caudal end of the mesothoracic cavity.

Metathorax (fig. c) : Comparatively simple ; prescutum invisible ; scutum represented by two lateral lobe-like processes, somewhat triangular with arms almost across ; anterior margin produced slightly to form the suralares ; the posterior margins form two knob like structures, the adanales ; scutellum small, narrow, situated in the hollow of the base of the scutum with lateral margins produced into membranous axillary cords extended upto the anal region of the hind wings ; postscutellum lying immediately caudad of the scutellum represented by a deep invagination, developed to a large phragma for muscle attachment.

Like the mesothoracic pleuron the metapleuron divided by a pleural suture into episternum and epimeron ; the epimeron ventrally fused with the basisternum and dorsally with a weakly sclerotized pad, the anterior basalare or the basalare pad ; the subalare situated in the membrane above a deep invagination in the dorsal margin of the epimeron ; the posterior region of the epimeron fused with the latero ventral extension of the postscutellum to form a postalare bridge deeply infolded and merged with the invagination of the postscutellum.

Basisternum narrow; furcasternum also narrow; the apophysis arising from the furcasternum bridge branched dorsally into an anterior and posterior pair of the furcal arms; the anterior arms shorter, the posterior arms much longer and fused with the ventral margin of the epimeron to form a narrow postcoxale.

Legs (Pl. V, figs. a, b, c, d e, & f)—The coxa of the prothoracic legs cylindrical, elongated, broad near the base and tapering towards the apex, articulated proximally with the trochantin; meso and meta-thoracic coxae immovably united with the pleura, but divided by a basicostal suture into an anterior eucoxa and a posterior meron; the latter produced distally into trochantifers articulating freely with the base of the trochanter; epicoxal piece broadly triangular.

The trochanter of all the legs subglobose; pro-and meso-thoracic trochanters equal in size; mesothoracic ones slightly shorter.

The femora of the pro-and meso-thoracic legs longer than the metathoracic femur; posterior margin of the prothoracic femur broadly rounded proximally, but tapered distally; ventral surface of the distal end deflexed for the accommodation of the epiphysis of the tibia under folded condition; dorsal surface provided with long cilia in both the sexes; male cilia thicker.

The tibia of the prothoracic legs (fig. a) short, stout and proximally provided with long spur, the epiphysis (epi); male epiphysis thicker and broader; mesothoracic tibia (fig. b) twice as long as the prothoracic ones and armed with a pair of spinous spur at the distal end; metathoracic tibia (fig. c) almost of the same length as those of the mesothoracic ones and armed with two pairs of spinous spurs.

The tarsus composed of five segments; the basitarsus longest, subsequent tarsi reduced in size progressively; distitarsus posteriorly provided with two rows of short stout spines ventrally; ungues (ung) most prominent; pulvilli at the base of the claws distinct; the area in between the claws much thickened representing the planta proximally and empodium distally; proximal end of the latter provided with three rows of sensory outgrowths on both the sides.

Wings (Pl. IV).

Forewing (fig. a) : Luteus, somewhat elongate, triangular, ramiform, orbicular with antemedial, medial and postmedial markings ; apical angle acute, anal angle obtuse ; inner margin slightly shorter than twice as long as the outer ; fringe light brown with a dark line at the base ; margins of the veins reddish ; costa fused with the costal margin ; subcosta free ; radius 1 and 5 unbranched ; 2, 3 and 4 stalked together ; media three branched, basal portion atrophid providing space for the big central cell ; cubitus two-branched, CuI bifurcated apically into CuIa and CuIb ; anal veins two, first anal missing, third anal recurved joining with second at midpoint.

Hind wing (fig. b) : Pale luteus with a dark brown stigma between the radius and the media ; broadly triangular ; outer margin twice as long as the inner ; apex broadly rounded ; fringe same as in the forewing, rather slightly lighter ; costa missing, probably converted into the frenulum hooks numbering one in the male and two in the female ; subcosta unbranched, basal two-third anastomosing with the unbranched radius ; media three-branched ; cubitus two-branched ; anal also two-branched, with a furrow in between A2 and A3.

Humeral plate very prominent, articulated with the costal vein ; first auxillary caudad of the humeral plate, articulated with the subcosta ; second axillary attached to the first, articulated with the base of the radius ; third axillary not clearly visible ; proximal median plate flat, semicircular ; caudad of the median plate, the basoplica knob-like.

Axillary sclerites of the hind wing practically the same as those of the forewing excepting the humeral plates ; the latter probably modified to a median plate at the base of the frenulum.

Abdomen and genitalia (Pl. VI, figs. a, b, c & d) : Abdomen moderately thick, consisting of ten segments ; the last two segments in the male and the last three segments in the female variously modified ; first abdominal segment much reduced ; tergite short, fused with the metathoracic plate ; the sternite fused with the sternite of the following segment forming a big lobe extended upto the ventral side of the third segment ; rest of the segments upto the eighth in the male and seventh in the female without any special modification ; the tergite of the eighth segment of the male tapered distally and beset with long hairs reaching caudad of the post genital segments ; the sternite of the same segment slightly reduced to make a greater space for the ventral region of the genital segment ; in the female the eighth sternite much modified and reduced to make a provision for the copulatory aperture, the ostium (os).

Male genitalia (figs. b and d): Male genitalia consisted of the dorsal tegumen, ventral vinculum, a pair of lateral clasping organs, the harpes and a median intromittent organ, the aedeagus ; tegumen tapered distally, posterior tip much hairy; vinculum 'V' shaped, anteriorly extended forword forming small bridge like structure, the succus ; the paired harpes somewhat spatulate and, excepting the basal region, the rest somewhat weakly sclerotized, inner surface concave and covered with long hairs, the ventral region or the sacculus produced into a curved inner process with a ventral prong separated from the upper region by a deep incission, dorsal margin partly serrate ; aedeagus long, tubular, eversible inside the thinly sclerotized anelus tube lying in between the two harpes.

Post genital segment or the anal chamber formed by a dorsal bulbous uncus and a ventral broad, thin, gnathos; anal opening situated in between these two plates.

Female genitalia (figs. a & c): Externally the female genitalia consists of the external opening of the ductus bursae or the ostium situated ventrad of the eighth segment, the true gonopore lying caudad of this, in between the two hairy lobes, the papilla aneles ; internally ductus bursae led to a big sac-like lobe, the corpus bursae ; vestibulum narrow ; ductus seminalis connecting the ductus bursae with the vestibulum and the short receptaculum seminis distinct.

Summary

The external morphology of the various stages of the Til Leaf Roller, *Antigastra catalaunalis* (Duponchel) has been studied in detail. The following are some of the salient features :—

Eggs broadly oblong, about 0.36 mm. long and 0.25 mm. broad ; pale transparent when laid, turning to red at the time of hatching.

The caterpillars of Ist to 5th instars measuring approximately 2.08 mm., 3.90 mm , 7.05 mm., 9.50 mm. and 15 mm respectively, in length ; breadth of the head capsule in the various instars—0.19 mm., 0.30 mm., 0.57 mm., 0.68 mm. and 1.62 mm. respectively ; ground colour dirty white

in the first instar, greenish yellow in the second and third, greenish in the fourth and fifth instars; in the fourth instar one pinaculum of the subdorsal region of the thoracic and abdominal segments 1-8 dark brown; in the fifth instar the dark brown pinaculi numbered three in the dorsal and subdorsal regions of each of these segments; ocelli six in number, ocellus 5 equidistant from the ocelli 4 & 6 and away from the ocellar ring; mandible six-toothed, lateral teeth on both the sides blunt, central teeth sharply pointed; spinneret long and narrow.

Pupae about 9.5 mm. long and 3.5 mm. broad; greenish when freshly formed, brownish at the time of emergence of adults.

Adults of moderate size; reddish yellow with antemedial, medial and postmedial reddish markings on the forewings; proboscis long; maxillary palpi prominent; fringe of the wing dark brown with a dark line at the base; frenulum present, numbering one in the males and two in the females; Cu2 present in the hind wings; tibia provided with long epiphysis; male epiphysis much thicker and broader; meso-and meta-thoracic tibia armed with one and two spinous spurs respectively; male genitalia situated on the 9th abdominal segment and consists of the dorsal tegumen, ventral vinculum, two lateral harps and a lateral intromittent organ; female genitalia of the ditrysian type, *i.e.*, with two genital openings, one for copulation and the other for oviposition; the copulatory aperture or the ostium situated ventrad of the 8th segment; the gonopore lying caudad of this and surrounded by the papilla aneles close and ventrad of the anus.

Acknowledgement

The authors are much indebted to Dr. B. P. Pal, the Director and Dr. E. S. Narayanan, The Head of the Division of Entomology, Indian Agricultural Research Institute, New Delhi, for their kind help and encouragement during the course of these investigations. Their sincere thanks are also due to Dr. M. G. Ramdas Menon, Systematic Entomologist, for critically going through the manuscript and making useful suggestions.

PLATE I

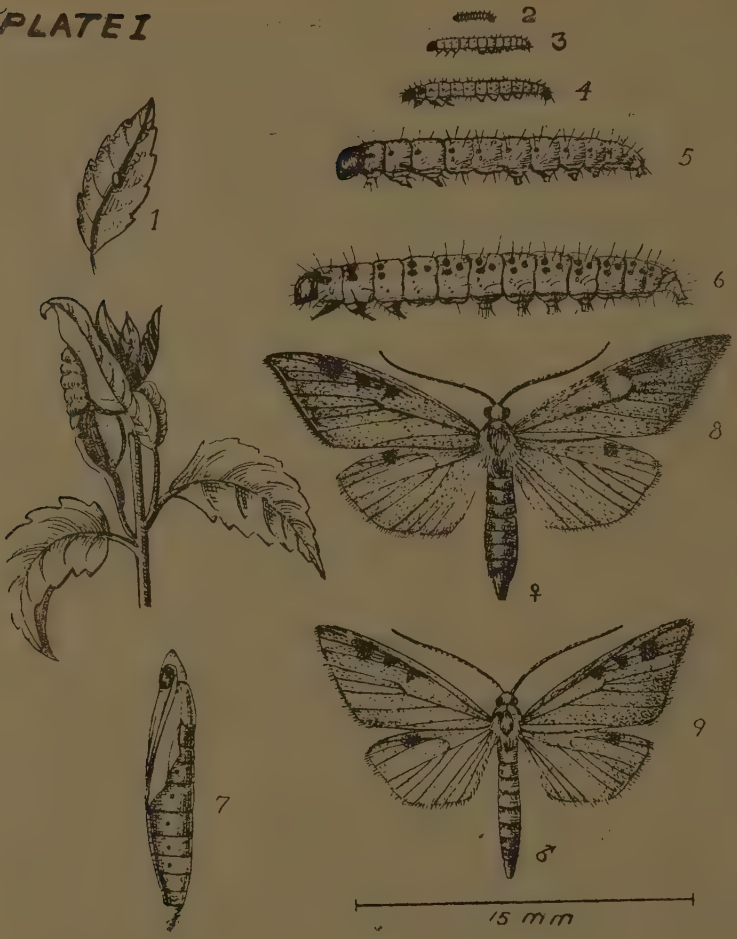

Plate 1 (Different stages)

Fig. 1. egg on the midrib of a leaf; fig. 2. first instar larva; fig. 3. second instar larva; fig. 4. third instar larva; fig. 5. fourth instar larva; fig. 6. fifth instar larva; fig. 7. pupa; fig. 8. female adult; fig. 9. male adult.

PLATE II

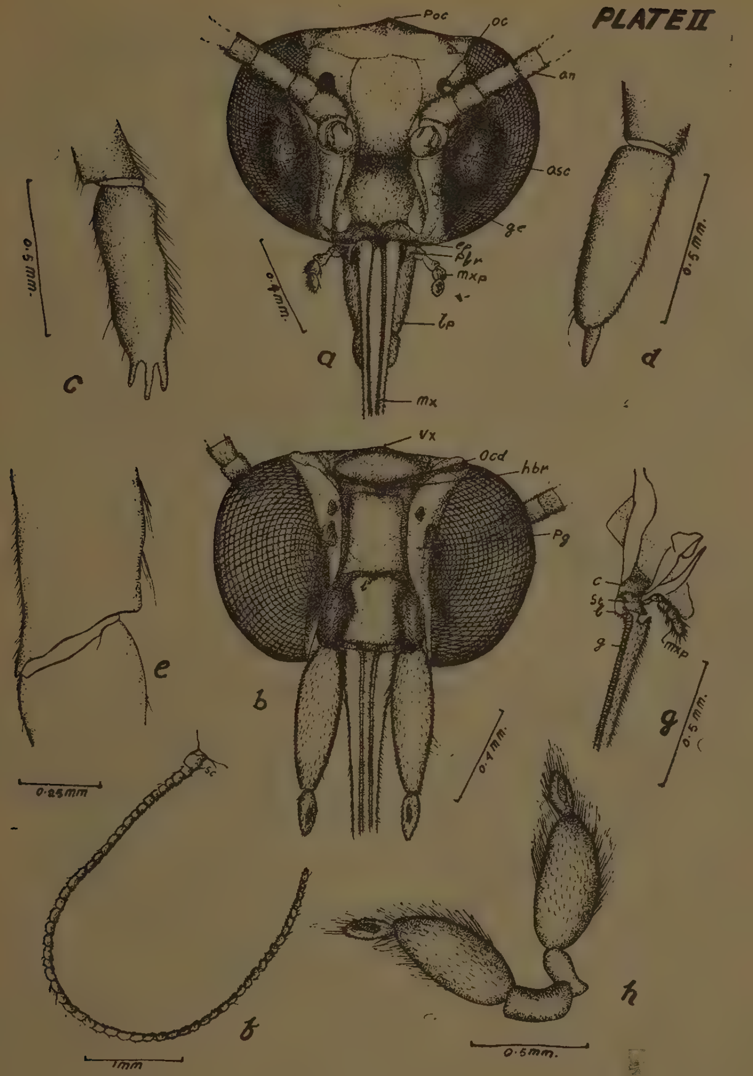

Plate II (Adult head and its appendages)

Fig. a—head capsule (anterior view); fig. b—head capsule (posterior view); fig. c—tip of the male antenna ; fig. d—tip of the female antenna ; fig. e—middle segment of an antenna, fig. f—entire antenna of a male ; fig. g—basal area of a proboscis ; fig. h—labial palpi.

an. antenna ; asc. antennal socket ; c. cardo ; g. galea ; ge. gena ; ep. epipharynx ; hbr. hypostomal bridge ; l'. labrum ; l. lacinia ; Lp. labial palpus ; pfr. palpiger ; pg. post gena ; oc. ocellus ; ocd. occipital condyle ; pg. post gena ; poc. postocciput ; v. vertex.

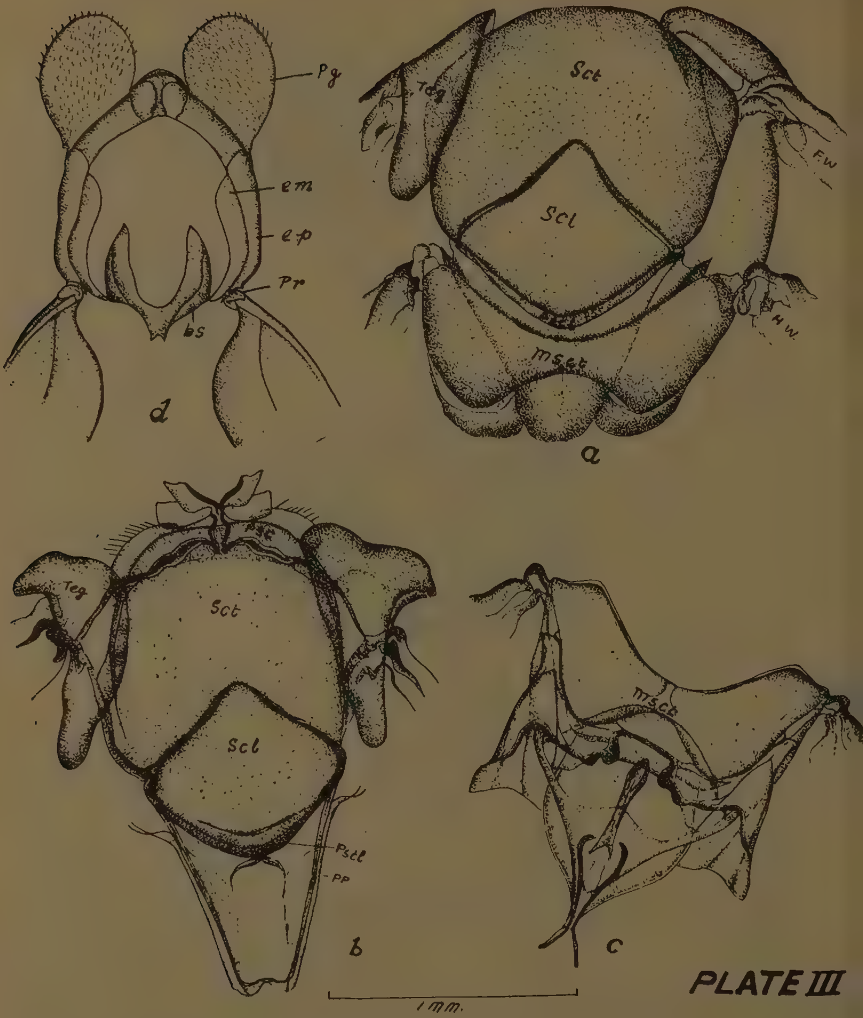

PLATE III

Plate III (Thoracic sclerites of an adult)

Fig. a—dorsal view of the mesothorax and metathorax; fig. b—dorsal view of the prothora and mesothorax; fig. c—dorsal view of the metathorax; fig. d—posterior view of the prothorax.

bs. basisternum; ep. episternum; em. epimeron; F.W. parts of the fore wing; H.W. parts the hind wing; msct. mesoscutum; pg. patagia; pp. phragma; pr. precoxale; psc. prescutum psct. post scutum; scl. scutellum; sct. scutum; teg. tegula.

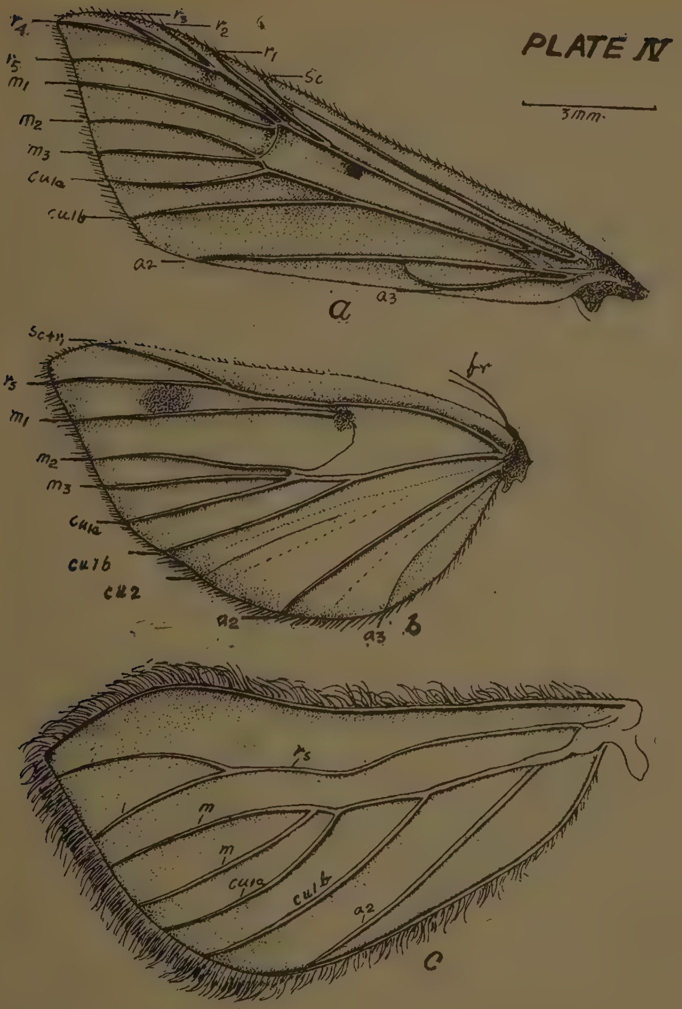

Plate IV (Wings).

Fig. a—fore wing; fig. b—hind wing of a female; fig. c—hind wing of a pupa.

a2 & a3. anal viens; cu1a, cu1b & cu2. cubital veins; fr. frenulum (female); m1, m2 & m3. medial veins; rs. radial sector; r 1-5. radial veins; sc. subcosta.

PLATE V

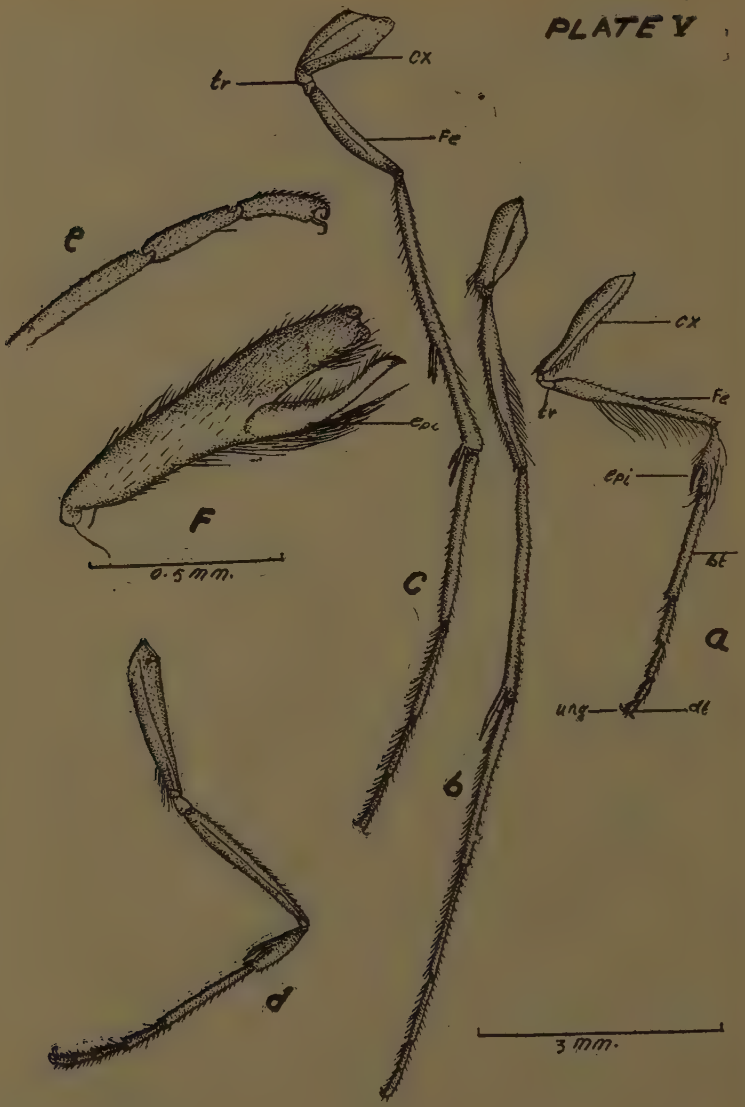

Plate V (Legs of adult)

Fig. a—fore leg (male); fig. b—middle leg; fig. c—hind leg; fig. d—fore leg (female); [fig. e—tarsal segments; fig. f—tibia.

bt. basitarsus; cx. coxa; dt. distitarsus; epi. epiphysis; fe. femur; tr. trochanter; ung. ungus.

PLATE VI

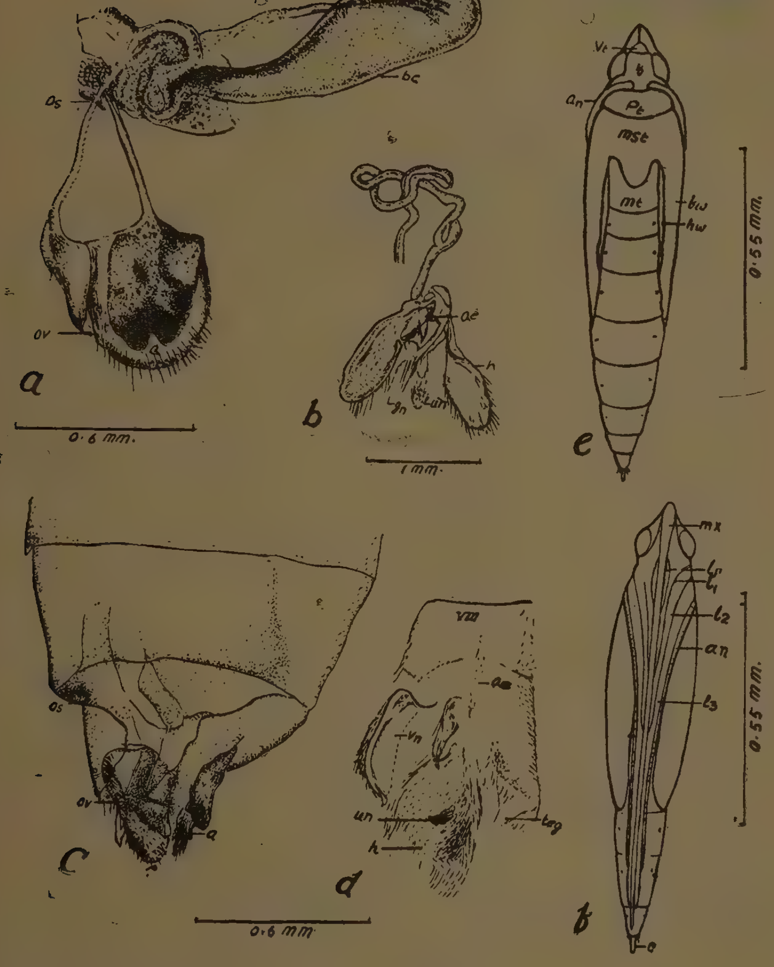

Plate VI (Adult genitalia and pupa)

Fig. a—female genitalia ; fig. b—male genitalia ; fig. c—genital segments of the female ; fig. d—genital segments of the male ; fig. e—dorsal view of the pupa ; fig. f—ventral view of the pupa.

a. anal opening; ae. aedeagus; an. antenna: bc. bursa copulatrix; c. cremastur; gn. gnathos; h. harpes; l1, l2, & l3 pupal legs; lp. labial palpus; h.w. hind wing ; mst. mesothorax; mt. metathorax; mx. maxilla; os. ostium; ov. ovipore; teg. tegula; un. ungus; vn. vinculum.

PLATE VII

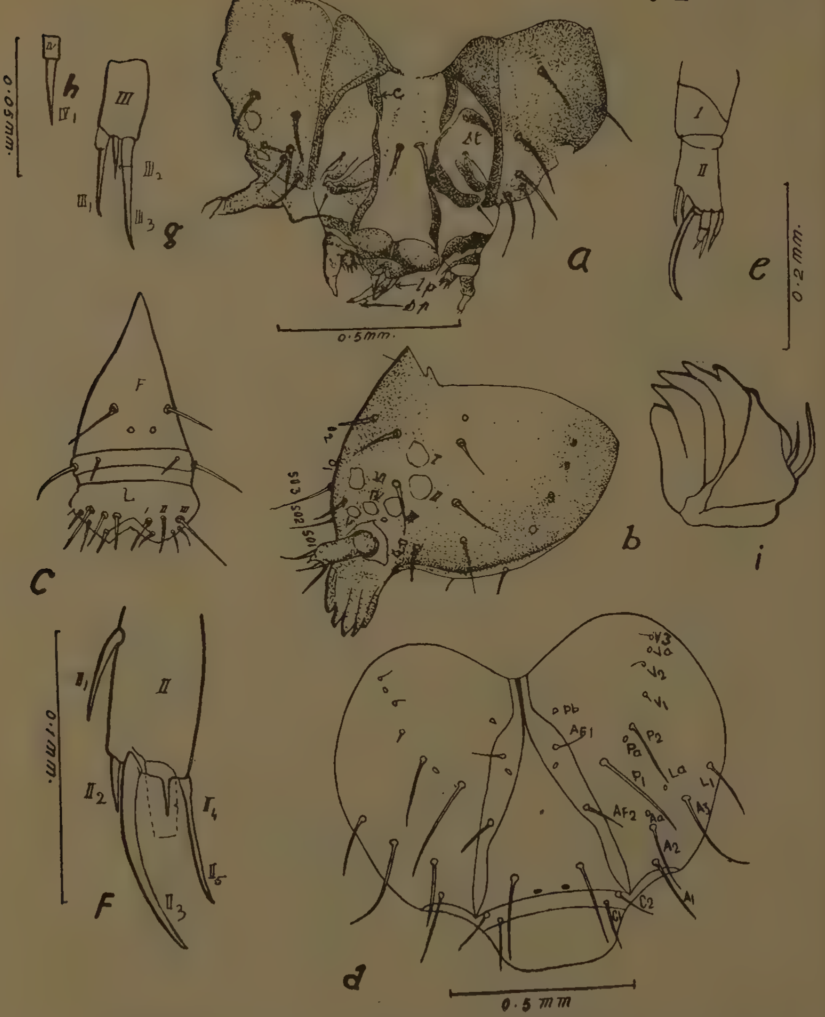

Plate VII (Chaetotaxy of the head capsule and the associated parts of a fully grown up caterpillar)

Fig a—hypopharynx complex; fig. b—lateral view of the head capsule; fig. c—frons, clypeus & labrum; fig. d—anterior view of the head capsule; fig. e—antenna; figs. f, g & h—second, third and fourth segments respectively of an antenna (enlarged); fig. i—mandible.

c. cardo; lp. labial palpus; sp. spinneret; st. stipes; A1–A3 anterior setae; Af1 & Af2 adfrontal setae; C1 & C2 clypeala setae; L1 lateral seta; La lateral puncture; O1–O3 ocellar setae; P1 & P2 posterior setae; Pa & Pb posterior punctures; V1–V3 microscopic setae of the vertex; Va puncture of the vertex.

Note:—In fig. b for O1 read O2, for O2 read O3, for O3 read O1.
In fig. d for AF1 read AF2, for AF2 read AF1.

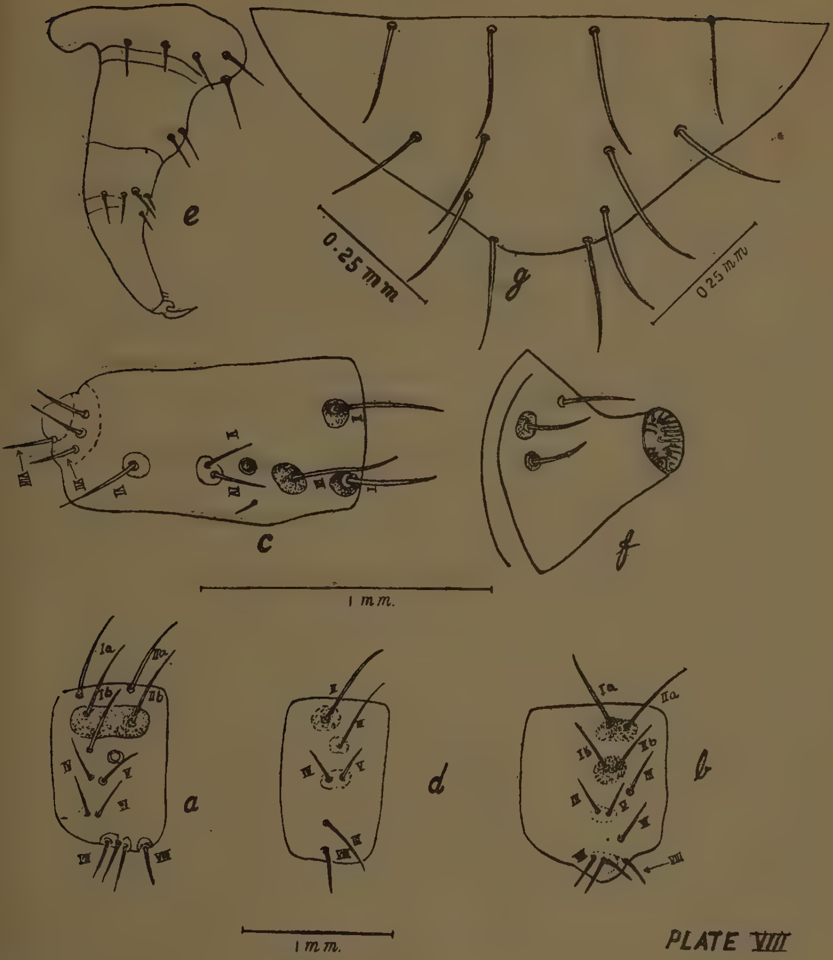

PLATE VIII

Plate VIII (Chaetotaxy of the thoracic and abdominal segments and their associated parts of a fully grown up caterpillar)

Fig. a—Prothoracic segment; fig. b—mesothoracic segment; fig. c—third abdominal segment; fig. d—ninth abdominal segment; fig. e—thoracic legs; fig. f—proleg; fig. g—anal shield.

Ia & Ib, IIa & IIb dorsal setae; III subdorsal setae; IV, V & VI subspiral setae; VII & VIII ventro lateral setae.

References

Dyar, A. G. 1894 Classification of lepidopterous larvae. *Ann. N.Y. Acad. Sci.*, **8** : 194-232

Forbes, W.T.M. 1910 A structural study of some caterpillars. *Ann. Ent. Soc. Amer.*, **3** : 94-132.

Forbes W.T.M. 1923 Lepidoptera of New York and the neighbouring states. Cornel Univ. agric. exp. stn., Memoir 68.

Fracker, C. 1915 Classification of lepidopterous larvae. *Illin. Biol. Monogr.*, **2** : 1-169.

Heinrich, C. 1916 On the taxonomic value of some larval character in Lepidoptera. *Proc. Ent. Soc. Wash.*, **18** : 154-164.

Hinton, H.E. 1945 On the homology and nomenclature of the setae of the lepidopterous larvae with some notes on the physiology of Lepidoptera. *Trans. Roy. Ent. Soc.*, London, **197** : 1-37.

Kapur, A.P. 1950 The identification of some Crambinae associated with sugarcane in India and of certain species related to them. *Trans. Roy. Ent. Soc.*, London, **101** : 389-434.

Ripley, L B. 1923 The external morphology and post embryology of noctuid larvae III. *Biol. Mongraph*, **8**(4).

Shephard, H H. 1930 The pleural and sternal sclerites of the lepidopterous thorax. *Ann. Ent. Soc. Amer.*, **23** : 237-246.

[Received for Publication on 25th March, 1960].

THE EXTERNAL MORPHOLOGY OF THE ANT-LION LARVA, *MYRMELEON SINGULARE* WESTW. (FAM. MYRMELEONTIDAE, NEUROPTERA.)

Part II—The Cervix, Thorax and Abdomen*

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The Cervix (Figs. 1 and 2)

The neck or cervix is a prominent portion. It is narrow, elongate and forms an anterior concavity in which the head capsule is lodged. Thus the posterior occipital area of the head is covered over by the neck.

There are two pairs of plate-like cervical sclerites of which the anterior pair is larger in size and triangular in shape. In the concavity of the anterior pair of sclerites fits in the head capsule; while in the concavity of the posterior pair of sclerites the anterior pair of sclerites are lodged. The posterior cervical sclerites articulate with the prothorax. Minute hairs are present over these sclerites. The dorsal and ventral regions of the cervix are membranous. The dorsal membrane extends over the head and articulates with it at two points around the foramen magnum. The dorsal levetor muscles of the head are seen to be attached from the occipital rim directly to the prothoracic tergum.

The neck can be extended or retracted. When the larva is at rest under the pit, the cervix is retracted and the head is bent below the thorax. It can be extended for catching the prey. Due to the peculiar muscular attachments of the head it can move upwards and can form a right angle with the neck when erected; but there is no sideways movement.

The Thorax (Figs. 1, 2 and 6A. B)

The prothorax is a small and narrow segment. The pronotum is differentiated into the prescutum, scutum and scutellum. The prescutum is a large, oval, shield-shaped plate. Anteriorly it extends over the posterior pair of the cervical sclerites, while posteriorly, it covers the scutum. Its margin is lined by small hairs. The scutum, which is partially covered

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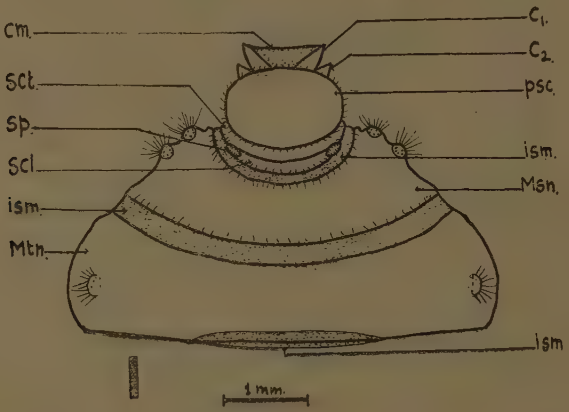
*Part I General description, the Head and its Appendages, appeared in vol, 7. no. 1 of this Journal.

[J. Anim. Morph. Physiol., Vol. 7, No. 2, pp. 125-133]

by the prescutum, has a semi-lunar shape. The scutellum is the smallest of the three sclerites. It is semi-lunar, and is closely applied to the scutum. Its posterior margin bears a row of hairs. At the outer margins of the scutellum, are present the large prothoracic spiracles. The spiracle is oval, surrounded by a thick peritreme. Its opening is traversed



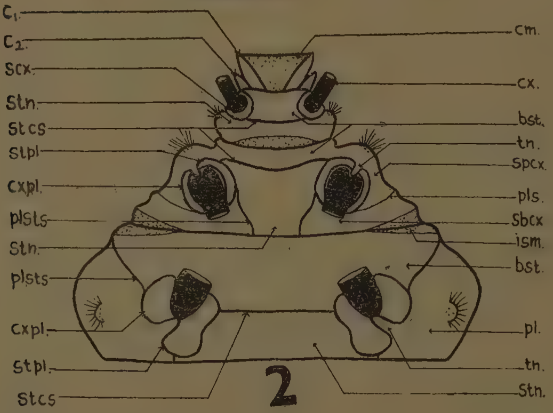


FIG. 1 Dorsal View of Cervix and Thorax.

C1—anterior cervical sclerite; C2—posterior cervical sclerite; cm—cervical membrane; ism—intersegmental membrane; Msn—mesonotum; Mtn—metanotum; psc—prescutum; scl—scutellum; sct—scutum; sp—prothoracic spiracle.

FIG. 2 Ventral View of Cervix and Thorax.

bst—basisternum; cx—coxa of leg; cxpl—coxopleurite; pl—pleuron; pls—pleural suture; plsts—pleuro-sternal suture; scx—subcoxal annulus; sbcx—subcoxal area; spcx—supracoxal area; stcs—sterno-costal suture; stn—sternellum; stpl—sterno-pleurite; tn—trochantin-like process.

through, by a number of fine chitinous, fringed rods, presenting the appearance of a grating, and thus apparently forming an air-filtering apparatus.

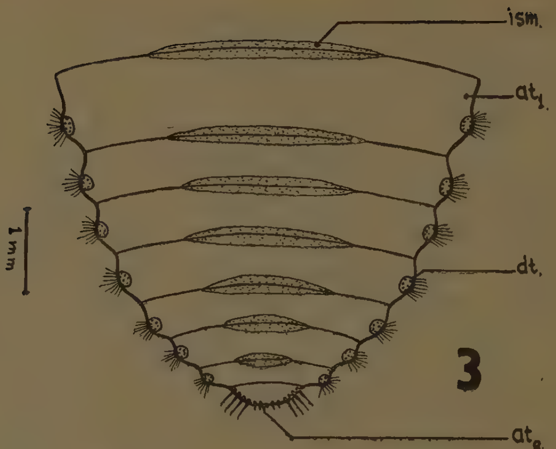
The pleuron is membranous and situated on the ventral side. There is no pleuro-sternal suture, and the pleuron is continuous with the sternum. The ventral sclerites thus form a compound sterno-pleural plate called the pectus. This is divided by the sterno-costal suture into two distinct plates, an anterior basisternum and the posterior sternellum. Anteriorly, the basisternum articulates with the cervical sclerites. The subcoxal ring is complete and raised over the general surface of the sternum. It is situated on the lateral sides of the basisternum. The sternellum is narrow in the midregion and broader at the lateral sides with a small protuberance carrying a group of long setae.

The intersegmental membrane between the pro-and mesothorax is broad on both the dorsal and ventral sides, as a result, the prothorax is freely movable ; a peculiar feature of the ant-lion larva.

The mesothorax has the shape of a trapezium ; the anterior portion is narrow, while the posterior region is broad. The anterior margin is deeply curved inside so as to form a large concavity in which the small narrow prothorax can be partly telescoped in. The posterior margin is slightly convex. Both the margins are lined by long hairs. The lateral sides, in the anterior half, show on each side, two protuberances beset with long spine-like setae.

The meso-tergum is an entire plate, but it is not fully chitinised. In the ventral view, the mesothorax shows a pleuro-sternal suture, separating the pleuron from the sternum. The pleural suture is not distinct. The sub-coxal annulus is formed of an outer (dorsal) coxopleurite and an inner (ventral) sterno-pleurite. On the dorsal side of the coxopleurite is the supra-coxal area, while below the subcoxal annulus is a large subcoxal area. Anteriorly, the subcoxal ring gives rise to a trochantin-like process which articulates with the coxa of the leg.

The sternum forms the mid-ventral region. The sternocostal suture is distinct and runs between the anterior margins of the subcoxal annulii ; and is seen to be continued down with the pleuro-sternal suture. The basisternum is a small, narrow band, forming the anterior region of the sternum in front of the subcoxal rings. The sternellum is a broad rectangular plate situated between the two subcoxal rings.



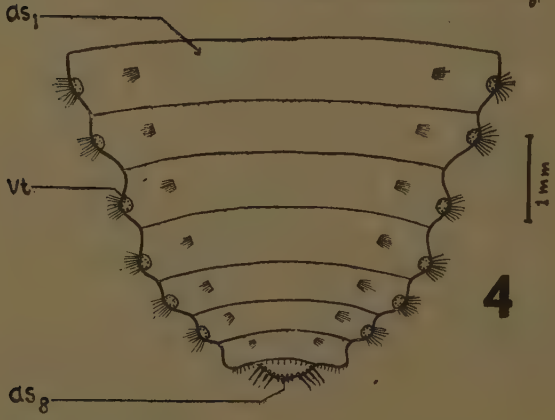


FIG. 3 Dorsal View of Abdomen.

at1 to at8—abdominal terga ; dt—dorsal tubercle ; ism—intersegmental membrane.

FIG. 4 Ventral View of abdomen.

as1 to as8—abdominal sterna ; vt—ventral tubercle.

The Metathorax is the largest and broadest thoracic segment. The metatergum is rectangular. Its lateral margins are convex and the anterior margin concave, while the posterior one is more or less straight. As a result, the metathorax is broader laterally and narrower medially. Laterally are situated a pair of protuberances carrying long spine like setae.

The pleuron is distinct from the sternum due to the presence of the pleuro-sternal suture which runs by the dorsal side of the subcoxal annulus. The pleural suture is absent and the supracoxal and subcoxal areas are fused. The subcoxal annulus is large and the coxo-pleurite and the sterno-pleurite elements are broad; between them is a very prominet trochantin-like projection articulating with the coxa. On each lateral side is a protuberance beset with long setae.

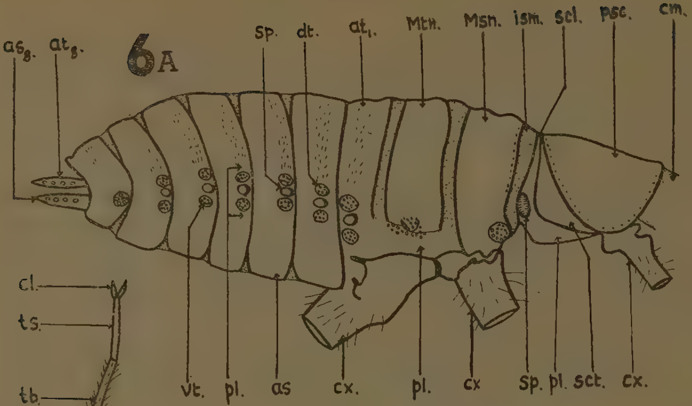
The sternum is a broad mid-ventral plate. The sterno-costal suture is distinct and runs between the sterno-pleurites of the subcoxal rings. The basisternum is a large plate, extending upto the lateral sides in the anterior region. The sternellum is a rectangular area between the sterno-pleurite.

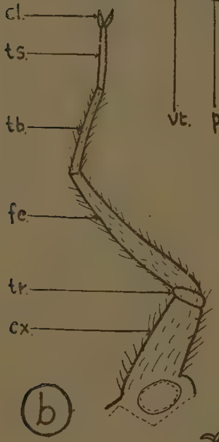
The intersegmental membrane between the meso-and meta-thorax is broad on the dorsal and lateral sides. In the mid-ventral region it is practically absent. The spiracles in the meso- and metathorax are absent.
The Legs: (Fig. 5, a, b, and c.)

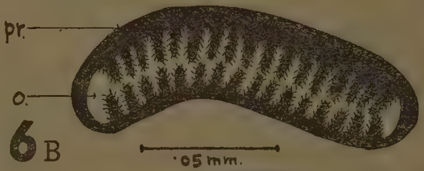
The prothoracic legs are long and slender, and directed forwards. Long hairs are present except on the last segment. The coxa is a long segment. The trochanter is a small, circular ringlike segment. The femur is the longest segment and has a more or less cylindrical shape. The tibia is also cylindrical but a little shorter than the femur. The last segment, the tarsus is slender, and a little shorter than the tibia. It is not divided into tarsomeres--a feature of common occurrence in holometabolous larvæ. The tarsus bears on its distal end a pair of delicate claws.

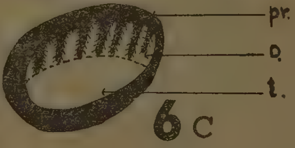
The mesothoracic legs are longer and stouter than the prothoracic ones and are laterally directed. All the segments except the last one, are clothed by long hairs. The coxa is broad and stout, tapering towards the distal end; the trochanter is ring-like; the femur is long, cylindrical and slightly curved; the tibia is slender, rod-like, and about half the length of femur; the tarsus terminates in a pair of small claws.

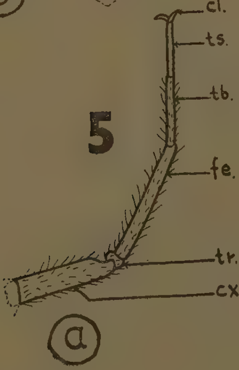
The metathoracic legs are shorter in length but stouter than the first two pairs. Unlike the prothoracic and the mesothoracic legs they are directed backwards. All its segments are hairy. The coxa is broad, rectangular in shape, and is as long as the femur; the trochanter is large, triangular; and wedged between the coxa and the femur in such a way that the femur is articulated at right angles to coxa; the femur is a stout. At the distal end the femur has two concavities. Next to the femur is a compound segment formed by the fusion of tibia and tarsus, the tibio-











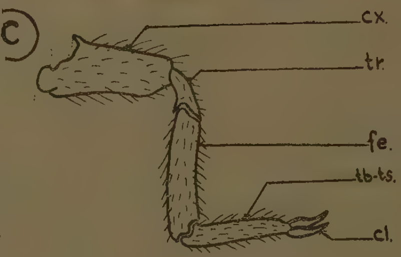


FIG. 5 The Legs.

a—prothoracic; b—mesothoracic, and c—metathoracic legs. cl—claws; cx—coxa; fe—femur; tb—tibia; tb-ts—tibio tarsal segment; tr—trochantor; ts—tarsus.

FIG. 6A Lateral View of Thorax and Abdomen.

The larva is cut in mid-line and spread laterally; the drawing is not to scale. Letters as in previous figures.

FIG. 6B prothoracic spiracle. 6C abdominal spiracle.

l—lid; o—opening; pr—peritreme.

tarsal segment. It is elongate and stout. Its proximal end has a dicondylic articulation with the femur—a characteristic of the larvae of Neuroptera and Trichoptera. At the distal end is a pair of large hollow and highly chitinised claws. They are globular at the base and curved at the tip.

Locomotion : The larva has a burrowing habit and after constructing the pit, it remains stationary at the base of the pit. When removed from the pit, it moves quickly in a retro-grade manner, by the rapid and forceful strokes of the legs. But when forced to move in the anterior direction, it hops with some difficulty using not only the legs but also the head and the mandibles. It's course backward in natural movement is not straight but somewhat curved. When disturbed, after moving a little, it retreats into the loose soil of the pit. It may then move under the soil, and construct a different pit.

The Abdomen (Figs. 3, 4 and 6A,B and C.)

The abdomen is clearly eight-segmented and cone-shaped. The first segment is as broad as the metathorax but the succeeding segments gradually get reduced in breadth towards the posterior end.

Primarily, the abdomen is dorsiventrally flattened, but it may assume a rounded shape when bloated. The intersegmental membranes on the dorsal side are clearly seen, but ventrally, they are not visible so well, as the sternites of segments are more close to each other. The terga and sterna themselves are soft and thinly chitinised. The identity between the terga, pleura and the sterna cannot be made out as the sutures are absent.

-The lateral sides are curved in the segments 1 to 7, so that at the intersegmental region there is a furrow while at the segmental region there is bulging.

There are two tubercles beset with long, thick setae, on the lateral sides of each segment in the pleural area (Fig. 6 A). One tubercle is situated dorsally and the other ventrally. Between the two tubercles is situated the abdominal spiracle. It is small, with a thick, oval peritreme. The opening is half closed by an incomlpete lid-like covering. On the opposite side of the lid are the fringed rods. The spiracle is very well guarded against any foreign particles by the two tubercles and their numerous setae which form a sort of a grating over the spiracle and completely hide it. This is an adaptation for utilising the air that is present in the loose soil. The setae at the same time prevent the blocking of spiracle by soil. The two tubercles and the spiracle between them is present on both the lateral sides of segments 1 to 6 only. In segment 7, there is only one tubercle on the lateral side. Abdominal spiracles are absent in the segments 7 and 8 (Fig. 6). Thus the larva has a peripneustic type of respiratory system in which the prothoracic spiracles and the abdominal spiracles are open ; those of the wing-bearing segments being closed.

The last or 8th segment is very small. The outer margins of the segment have setae of two types ; the middle setae are short, blunt, tubercle-like ; while the lateral setae are long and spine-like. This segment bears the anus.

Ventrally, in the segments 1 to 7, each sternum has a group of elongated hairs forming a comb-like structure on either side of the mid-region. The 7th segment also bears on its posterior margin a row of stiff hairs. Dorsally, in segments 1 to 7, each tergum shows a narrow belt of minute hairs in the middle region and extending upto the lateral sides.

It has been observed that, the last two abdominal segments with their peculiar arrangment of long, stiff setae and hairs, are used to dig out the pit of the larva. The larva enters the soil by the posterior end, dislodging the soil the help of these segments.

Summary

1. The cervix, the thorax and the abdomen are described in detail.
2. The prothorax is narrow, and with an oval pronotum. It is markedly different from the broad, rectangular mesothorax and metathorax.
3. The legs are articulated to the thoracic segments each by a subcoxal annulus. The first two pairs of legs are slender, the prothoracic ones forwardly directed, and the mesothoracic ones laterally directed. The third pair of legs are stout, backwardly directed, and with a compound tibio-tarsal segment having dicondylic articulation with the femur. The locomotion is described in detail.
4. The abdomen is cone-shaped, with thinly chitinized, eight ring-like segmental plates, and with lateral tubercles beset with setae.
5. The respiratory system is peripneustic, their being only a single pair of prothoracic and six pairs of abdominal spiracles.

Acknowledgment

The author is thankful to Principal P. R. Damle, and Dr. D. N. Kamat, Head of the Department of Zoology, N. Wadia College, Poona, for the permission and facilities given by them to carry out the work in the College Laboratory. The author is particularly thankful to Shri D. R. Ranade, Lecturer in Zoology, and Entomology, Department of Zoology, University of Poona, for the suggestion of the problem, guidance and for going through the manuscript.

References

Chitnis, P. S. 1960 The external morphology of the Ant-lion larva, *Myrmeleon singulare* Westw. Part I. *J. Anim. Morph. Physiol*, 7, 1.

[Received for publication on 10th June, 1960.]

GLYCOGEN FRACTIONS IN THE CONDUCTING SYSTEM OF THE OX HEART

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Kolmer and Fleischman (1927), Buadze and Wertheimer (1928) and Murray (1951), in respiration studies with mammalian cardiac 'conducting tissue' (Neuromyocardium), have found that it has a particularly low oxygen uptake as compared with other tissues, and Clark and Kingisepp (1935) found similar low oxygen uptake in the sinus tissue of the frog. Wearn (1928), in a series of experiments with human hearts, found the capillary circulation to the conducting system to be approximately half that of the circulation to the myocardium. The indifference of the conducting system to oxygen lack may be further illustrated by Wilson's (1936) observation that the Purkinje network is very resistant to anoxaemia and may remain alive after all the surrounding muscle has been killed by ligation of the vessel which supplies it.

Post-mortem autolysis causes glycogen to disappear very quickly in ordinary heart muscle (myocardium), but F. Davies (Personal communication) noted that it disappeared much more slowly in the large Purkinje fibres of the 'conducting system' of the ox heart. Chemical estimations by Yamazaki (1929) on the horse and ox, by Yater, Osterberg and Hefke (1930) on the horse, and by Noll and Becker (1936) on the horse and calf have shown that there is more 'total glycogen' in Purkinje fibres than in the myocardium. The concept that glycogen exists in the tissues in two forms, namely fixed to protein (desmoglycogen) and free, acid-soluble glycogen (lyoglycogen), has been reviewed by recent workers (Bloom, Lewis, Schumpert and Shen, 1951; Merrick and Meyer, 1954; Russell and Bloom, 1955; Merrick, 1957; Weisberg and Rodbard, 1958). It is believed that lyoglycogen is the more labile and that it is probably the metabolically active form. Of the two glycogen fractions present in cardiac tissues of the dog, Merrick (1957) observed that the unbound fraction was drastically reduced under conditions of anoxia.

In the present work, both the total glycogen and the lyoglycogen

[J. Anim. Morph. Physiol., Vol. 7, No. 2, pp. 134-140, 1960]

content of these tissues in the ox heart has been estimated and the difference in both desmoglycogen and lyoglycogen content thus determined. An explanation for the apparent indifference of Purkinje fibres to oxygen lack has been attempted on the basis of these results.

Materials and Method

Ten ox hearts were obtained from the public abattoir within 5 minutes of the beasts being slaughtered. The left ventricle was opened and a piece of the heart muscle was removed and quickly frozen in dry ice. The atrioventricular bundle pierces the os trigonum dextrum and then divides into left and right limbs which run under the endocardium of the interventricular septum. The major part of the right limb leaves the interventricular septum and passes into the moderator band. The left or right limb was quickly dissected free from the os trigonum dextrum and from the underlying muscle of the interventricular wall or moderator band. A section approximately 0.25 cm. was removed from the centre of this strip, which was about 4 cm. long, and fixed in ice cold 80% alcohol. The remaining parts were frozen in dry ice. The remainder of the heart was kept at room temperature for six hours when further specimens of myocardium and Purkinje fibre were taken for glycogen estimation.

The total and the acid soluble glycogen content of the frozen myocardium and Purkinje fibres was estimated chemically (Kugler and Wilkinson, 1960). The fixed specimens of Purkinje fibres were dehydrated, cleared, embedded in paraffin wax and sectioned at 8μ. Serial transverse sections of the conducting tissue were stained with iron haematoxylin and van Gieson stain to demonstrate connective tissue. The proportion of Purkinje fibres to connective tissue and myocardium in selected slides was determined with a planimeter and it was assumed that these specimens were representative of the whole.

Results

The amounts of total glycogen and acid soluble glycogen present in both myocardium and conducting tissue are expressed in μg/100 mg tissue wet weight. These values in respect of the conducting tissue have been corrected for amount of "diluent material" (connective tissue and myocardium) present in each specimen. On the average, 30% of the total specimen was Purkinje fibre but the percentage present in one case was 45% and in another as low as 10%. This variation in the amount of

TABLE I

	Total Glycogen (µg/100 mg. tissue, wet weight)				Acid Soluble Glycogen (µg/100 mg. tissue, wet weight)			
	Myocardium		Purkinje Fibre		Myocardium		Purkinje Fibre	
Time (hours) post-mortem	0	6	0	6	0	6	0	6
Animal Number								
1	724	257	21,430	658	360	44	8,820	304
2	341	117	9,463	2,695	123	0	7,111	1,350
3	223	148	6,884	4,077	54	0	6,450	1,826
4	361	132	1,694	493	92	0	880	444
5	522	264	1,529	⁂	329	66	750	⁂
6	659	291	1,951	⁂	464	12	1,010	⁂
7	266	112	5,234	2,206	66	0	642	1,660
8	359	96	3,575	3,683	77	6	2,070	1,027
9	358	89	1,932	1,241	117	0	306	574
10	358	98	1,802	1,322	195	0	573	750

⁂ No Purkinje fibres were found in these specimens on histological examination.

TABLE II

Percentage of acid soluble glycogen
(Lyoglycogen)

Tissue	Time post-mortem	%
Myocardium	0 hours	45
Myocardium	6 hours	7
Bundle—Purkinje Fibre ..	0 hours	51
Bundle—Purkinje Fibre ..	6 hours	49

conducting tissue in each specimen has been previously noted by Murray (1954). All the results are expressed in Table 1 and the percentage of lyoglycogen present in myocardium and conducting tissue five minutes post-mortem and six hours later is expressed in Table 2.

There is considerable difference in the glycogen content of the individual hearts as it was impossible to control such factors as breed, weight, age and nutritional states of the experimental animals. When the series is reviewed as a whole the very high total glycogen content of the Purkinje fibre as compared with that of the myocardium is striking; this finding being in agreement with previous workers (Yamazaki, 1929; Yater *et al.*, 1930; Noll *et al.*, 1936). Although the total glycogen content of the Purkinje fibre fell after six hours breakdown, the percentage of lyoglycogen fell only 3% from its initial value of 51% of the total during this period. Of the small quantity of glycogen present at first in the myocardium, 45% was lyoglycogen, but after six hours glycogenolysis this fraction comprised only 7% of the remaining glycogen.

Discussion

Skeletal muscle contains large quantities of glycogen. It may undergo aerobic or anaeobic degradation with the formation of high energy phosphate bonds for resynthesis of the adenosine triphosphate depleted during contraction. Owing to the inability of the circulation to meet the increase requirement for oxygen, increasing amounts of lactic acid are produced from the breakdown of glycogen. Nervous tissue contains very little carbohydrate; it obtains glucose for its metabolic processes direct from the arterial circulation and has very small reserves upon which to draw in cases of emergency, although this reserve is not affected by other factors which profoundly influence liver glycogen *e.g.* starvation and adrenaline. Nervous tissue contains all the enzymes and metabolic intermediaries for anaerobic and aerobic carbohydrate metabolism, but anaerobic metabolism of glucose cannot supply the energy requirements for normal cerebral and nervous function. This is reflected in the comparatively high oxygen consumption of the brain, which at times can be as much as 25% of the total basal body requirements.

Merrick and Meyer (1954) observed that there was a species difference in the amount of acid soluble glycogen present in hearts and that lyoglycogen was drastically reduced under conditions of anoxia. Of the glycogen present, all was acid soluble in goldfish hearts, 87% acid soluble in dog hearts, 55% was acid soluble in rat hearts and only 19% of the total was acid soluble in mouse hearts. The species that had the greater quantity of lyoglycogen present were the most resistant to anoxia. Thus dog and rat hearts, though less susceptible than the mouse, survive a much shorter time under low oxygen tensions than that of goldfish.

From the percentage expressed in Table 2 it is manifest that there is great decrease in the amount of lyoglycogen in myocardium after six hours under anoxic conditions, while there is a very small drop in the unbound fraction in Purkinje fibres. This difference between the two may be important. The reduction of the unbound fraction in the myocardium under these conditions may be a mechanism of survival value in transient states of oxygen depletion—but this will be very limited, as under these conditions glycogen is not reformed; thus the whole system will be sensitive to more than short periods of anoxia. On the other hand, the very slight fall after 6 hours in the unbound fraction in conducting tissue may indicate that it is resistant to anoxic conditions, this being in direct contrast to the work of Hoff (1949).

Sodium and potassium ions are known to be concerned with the transmission of impulses along peripheral nerves. During the recovery phase there are movements of these ions against concentration gradients which require large amounts of energy. It is widely believed that the sinu-atrial node, the atrioventricular node and the atrioventricular bundle with its left and right limbs are concerned with the initiation and transmission of the cardiac impulse to the constituent parts of the heart (Davies, Francis, Wood and Johnson, 1955). Although the exact mechanism of transmission of the cardiac impulse is not known, Davies, Davies, Francis and Whittam (1952) found the distribution of sodium and potassium ions in conducting tissue to be similar to that in peripheral nerves. If the mechanism of transmission of the cardiac impulse along the conducting system is the same as that along peripheral nerves it may be that the large reserves of glycogen in Purkinje fibres are concerned with these energy requirements.

The results expressed in the present paper indicate that the conducting system of the ox heart has large reserves of glycogen which, under conditions of anoxia, appear to react differently from similar reserves in the myocardium, and the katabolic processes involved may afford the conducting system some degree of protection in anoxic conditions.

Summary

Glycogen fractions in myocardium and in conducting tissue of the ox heart have been estimated chemically for a certain period of time post-mortem. Myocardium contains some reserve of glycogen but this is severely depleted in anoxic conditions. The conducting tissue contains a large amount of glycogen initially and six hours post-mortem. Glycogen may be concerned with the transmission of the cardiac impulse and the difference in glycogenolysis may afford the conducting system some degree of protection in anoxic conditions.

References

Bloom, W. L., Lewis, G. T., Schumpert, M. Z. and Shen, T. 1951. Glycogen fractions of liver and muscle. *J. biol. Chem.*, **188**: 631-636.

Buadze, S. and Wertheimer, E. 1928. Der Glykogengehalt des Reizleitungssystems im Herzen. *Pflug. Arch. ges. Physiol.*, **219**: 233 237.

Clark, A. J. and Kingisepp, G. 1935. The effect of low oxygen pressures on frog's cardiac tissue. *Quart. J. Exp. Physiol.*, **25**: 279-302.

Davies, F., Davies, R. E., Francis, E.T.B. and Whittam, R. 1952. The sodium and potassium content of cardiac and other tissues of the ox. *J. Physiol.* (Lond.), **118**: 276-281.

Davies, F., Francis, E.T.B. Wood, D. R. and Johnson, E. A. 1955. The atrioventricular pathway for conduction of the impulse for cardiac contraction in the dog. *Trans. roy. Soc.* Edinb., **63**: 71-84.

Hoff, H. E. 1949. A Text-book of Physiology. Ed. J. G. Fulton : Saunders : Philadelphia : P. 644.

Kolmer, W. and Fleischman, W. 1927. Uber den Sauerstoffverbrauch der Purkinjeschen Faden. *Pflug. Arch. ges. Physiol.*, **218**: 530-531.

Kugler, J. H. and Wilkinson, W. J. C. 1960. Glycogen fractions and their role in the histochemical detection of glycogen. *J. Histochem. Cytochem.*, **8**: 195-199.

Merrick, A. W. and Meyer, D. K. 1954. Glycogen fractions of cardiac muscle in the normal and anoxic heart. *Am. J. Physiol.*, **177**: 441-443.

Merrick, A. W. 1957. Experimental coronary occlusion in dogs and its effect upon cardiac glycogen fractions. *Circulation Research*, **5**: 435-437

Murray, J. B. 1954. Oxygen uptake of the atrioventricular conducting tissue of beef heart. *Am. J. Physiol.*, **177**: 463-465.

Noll, A. and Becker, M. 1936. Der Glycogengehalt der Musculatur und des Erregungsleitungs-systems des Herzens nach chemischen und microskopischen Untersuchungen. *Virchows Arch. path. Anat.*, **296**: 443-447.

Russell, J. A and Bloom. W. L. 1955. Extractable and residual glycogen fractions in tissues of the rat. *Am. J. Physiol.*, **183**: 345-355.

Wearn, J. T. 1928. The extent of the capillary bed of the heart. *J. Exp. Med.*, **47**: 273-291.

Weisberg, J. and Rodbard, S. 1958. Distribution of glycogen in the rat heart. *Am. J. Physiol.*, **193**: 466-468.

Wilson, F. N. 1936. Diseases of the Coronary Arteries and Cardiac Pain. Ed. R. Levy: Macmillan: New York: P. 316.

Yamazaki, K. 1929. Biochemical studies on the auriculo ventricular system of t e heart. I. The glycogen content. *J. biol. Chem.*, **10**: 481-490.

Yater, W. M., Osterberg, A. E. and Hefke, H. W. 1930. Chemical determination of the glycogen ratio in the bundle of His and the cardiac muscle in man and the mouse. *Arch. Intern. Med*, **45**: 760-771.

[Received for publication on 12th November, 1960]

THE FIBRE DIAMETER AND CERTAIN ENZYME CONCENTRATIONS IN THE FLIGHT MUSCLES OF SOME MOTHS

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(2 Plates)

By virtue of the structural peculiarities associated with corresponding functional diversity, the striated muscle of insects could be considered a good material for studies directed towards a better understanding of the relationship between structure and function Marked differences in the metabolism of the muscles of different insects have been found to exist and could be attributed to the differences in structure and mode of action of the muscles concerned. Differences in the tracheation, colour and variations in the ultra structure of the isolated fibrils in insects are also known (Edwards, Santos, Santos and Sawaya, 1954a, b). However, little is known regarding the differences in the nature of the fuel store in different muscles and their bearing on the mode of flight though it has long been an established fact that grasshoppers lay down large store of fat before making migratory flights and in the case of the beet leafhopper it has been suggested (Fulton and Romney, 1940) that its fat content is an index of its flying capacity. Krogh and Weis-Fogh (1951) and Weis-Fogh (1952) have shown that the respiratory quotient of the desert locust *Schistocerca gregaria*, during sustained flight equals to 0.74 – 0.75 which is indicative of fat utilization. The Lepidoptera seem to be even more specialized in this direction. They do not seem to utilize glucose directly to a significant extent but convert it into fat before utilization (Zebe, 1954, 1957). Obviously our recent work (1960) demonstrating the presence of considerably high concentrations of a fat-splitting enzyme (lipase) in the flight muscles of some butterflies indulging in sustained flight is of special significance. The results of similar studies conducted in our laboratories on the flight muscles of the various flying animals such as the pigeon, bat, locust and dragon fly have also shown the presence of high concentrations of fat and lipase in these muscles (George and Naik,

[J. Anim. Morph. Physiol., Vol. 7, No. 2, pp. 141-149, 1960]

1958a, b, 1959a, b; George and Scaria, 1956; George, Susheela and Scaria, 1958; George, Vallyathan and Scaria, 1958). It has been pointed out that in these muscles there exists a correlation between the lipase content and fibre diameter—greater the diameter lesser the lipase content and lesser the diameter greater the lipase content. It was also shown that the oxidative enzymes are concentrated more in narrow fibres than in broad ones. It was therefore thought desirable to carry out similar investigations on the flight muscles of some moths and the results obtained are presented here.

Materials and Methods

The flight muscles of the following moths were examined.

Family	Name
Sphingidae	*Cephonodes hylas hylas* (Linn.)
,,	*Polyptychus dentatus* (Cramer)
,,	*Hippotion rafflesi* (Butl)
,,	*Hippotion celerino* (Linn)
,,	*Deilephila nerii* (Linn.)
,,	*Herse convolvuli convolvuli* (Linn)
,,	*Acherontia styx styx* (Westw.)
Noctuidae	*Ophideres meterna* (Linn)
,,	*Nyctipao macrops* (Linn)

All the moths studied were caught at night during flight except *Cephonodes hylas hylas* which was caught during flight during the day and used after killing them by decapitation. The thorax was cut open on the ventral side and after pulling out the alimentary cannal the muscles were carefully removed from their attachment by a pair of fine watchmaker's forceps and used for the different observations.

A. *Measurement of fibre diameter*

Very thin hand sections of fresh frozen muscle, cut according to the method of George and Scaria (1958a) and mounted in glycerine jelly, were used for the measurement of fibre diameter. A microscope with occular eye piece and micrometer scale was used for the measurement.

B. *Enzymes*

1. *Quantitative*

The concentration of lipase in the flight muscle of the above moths was studied quantitatively according to the method of Martin and Peers

(1953) using the conventional Warburg apparatus, in a bicarbonate-carbon dioxide buffer system of pH 7.4 at 37°C using tributyrin as substrate. The reaction flask contained 1.5 ml of 0.025 M bicarbonate solution and 1 ml of the enzyme solution in the main chamber and 0.5 ml of 4% (v/v) tributyrin in 0.0148 M bicarbonate (emulsified by shaking with a drop of " Tween 80 ") in the side arm, thus making up the total volume of 3 ml in the reaction flask. The manometer and flask were gassed for 3 minutes with a mixture of 95% nitrogen and 5% carbon dioxide. After equilibration for 10 minutes in the constant water bath of the apparatus, the substrate was tipped in and after 3 minutes the initial readings were noted. Readings were taken twice in an hour.

Lipase activity was calculated on the basis of the protein concentration of the enzyme solution used and is expressed as the number of μl CO_2/mg. protein/hr. Protein was estimated according to the micro-Kjeldahl steam distillation method (Hawk, *et al*, 1954).

2. *Histochemical*

The localization of the following enzymes were studied histochemically; lipase, acid and alkaline phosphatases, adenosine triphosphatase (ATPase) and succinic dehydrogenase.

Fresh frozen sections of the muscles prepared according to the method described by George and Scaria (1958a), were used for the histochemical studies. Lipase was studied according to the method of Gomori using " Tween 80 " as substrate at a pH of 8.4. The revised method of Gomori using sodium glycerophosphate as substrate, was employed in the study of alkaline phosphatase at a pH of 9.2 (Pearse, 1954). Acid phosphatase was tested according to Gomori's revised method (Glick, 1949) using sodium glycerophosphate as substrate at pH 5. The procedure was the same as described by Glick (1949). In all the above experiments sections kept in boiling water for 10 minutes prior to incubation were used as control. Sections were incubated for 6 to 8 hours for lipase and 24 and 6 hours for alkaline and acid phosphatases respectively at 37°C. The presence of ATPase was tested by employing the method of Pearse and Reis (Pearse, 1954) using adenosine triphosphate (ATP) as substrate. Sections were incubated for 3 hours which was found to be the optimum period for these muscles. The following were the controls employed: sections for alkaline phosphatase incubated at pH 9.2 and 7.5; sections kept in boiling water for 10 minutes before incubation.

Succinic dehydrogenase activity in the muscles was studied following the method of Straus, *et al* (Pearse, 1954) using 2 : 3 : 5 triphenyl tetrazolium chloride (TTC) as the hydrogen acceptor. The procedure was exactly as described by George and Scaria (1958b).

Results

A. *Fibre diameter*

The average diameter of the fibres corrected to the nearest whole number, in the different species is given in Table 1. It was observed that the fibre diameter of the muscle was more or less uniform for the species. The largest as well as the smallest fibres were found in the Sphingidae ranging from 75 to 103 μ while in the other family studied (Noctuidae) the fibres were of more or less the same diameter (85-86 μ). The figures given for diameter represent the average of several hundred fibres measured.

B. *Lipase activity of the muscles*

The results of the study made on the quantitative determination of lipase activity in the flight muscles of the different moths are presented in Table 1. It can be noticed that the greatest activity of 31.76 μl CO_2/mg. protein/hr. is in the sphingid moth *Cephonodes hylas hylas* which possesses the narrowest fibres and the least (10.96 μl CO_2/mg protein/hr.) also in a sphingid moth *Acherontia styx styx* but having the largest fibres.

C. *Histochemical observations*

(1) *Lipase*

The flight muscles of all the moths studied gave a positive staining reaction for lipase. However clear histochemical distinction between those showing greater or lesser activity was possible only in those that formed the highest and lowest amounts of the precipitate obtained in the two species with smallest and largest fibres respectively. The quantitative study revealed the exact differences in the level of lipase activity in them.

(2) *Phosphatases*

All the phosphatases tested for, namely acid and alkaline phosphatases and adenosine triphosphatase could be demonstrated histochemically in the flight muscles of all the moths studied. The localization of the enzymes was uniform in the fibres For ATPase a very dark colour was obtained in all the cases denoting a very strong positive reaction for the enzyme.

(4) *Succinic dehydrogenase*

The colour developed due to formazan was uniform in all the fibres of the flight muscles in all the moths observed. A quantitative estimation of this enzyme would be more confirmatory in assessing the relative differences.

TABLE I

Lipase activity in the flight muscles of some moths studied and their average fibre diameter. The value given for the lipase activity is the average of 5 experiments in each case.

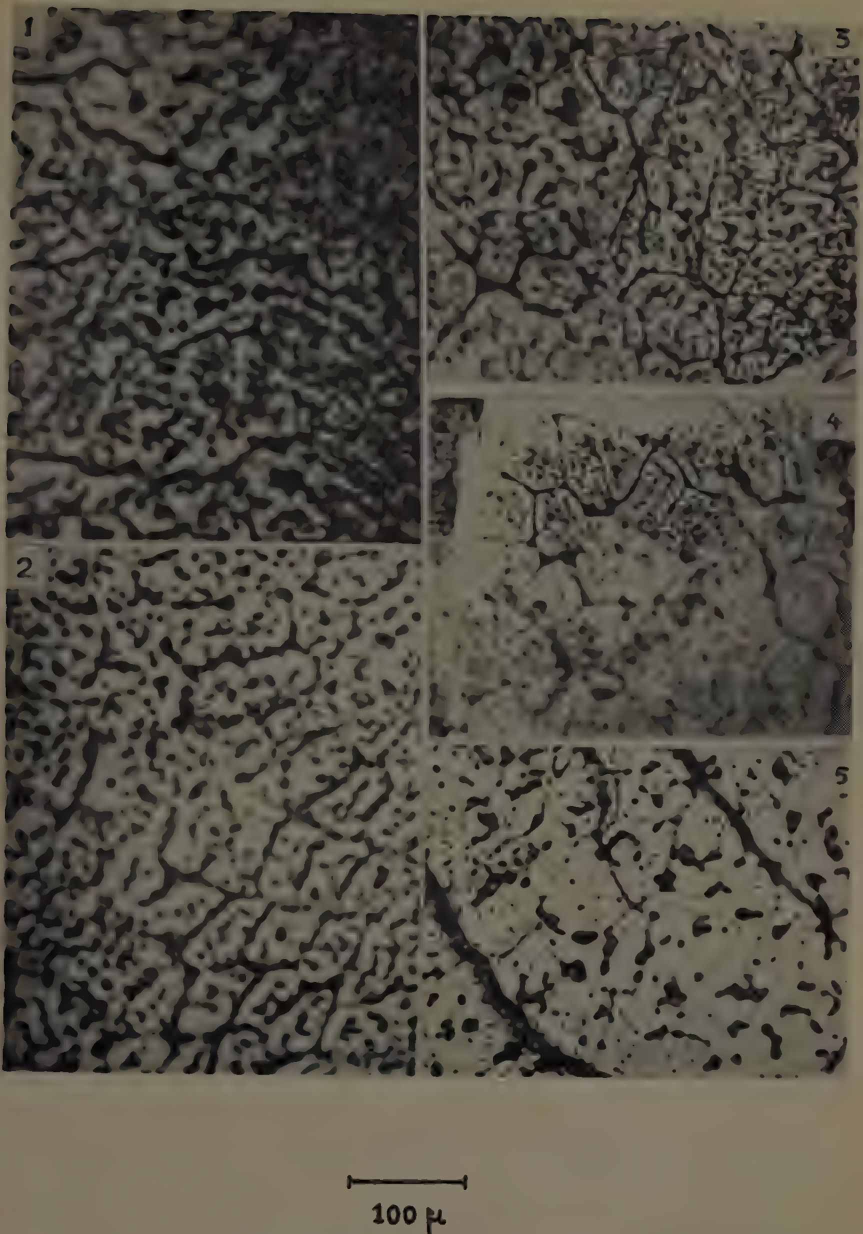
Family	Name	Lipase activity μlCO_2/mg. protein/hr.	Average fibre diameter μ
Sphingidae	*Cephonodes hylas hylas* (L)	31.76	75
,,	*Polyptycus dentatus* (Cram)	31.74	75
,,	*Hippotion rafflesi* (Butl)	26.81	80
,,	*Hippotion celerino* (L)	24.20	82
,,	*Deilephila nerii* (L)	20.23	86
,,	*Herse convolvuli convolvuli* (L)	15.37	95
,,	*Acherontia styx styx* (L)	10.90	103
Noctuidae	*Nyctipao macrops* (L)	23.90	85
,,	*Ophideres materna* (L)	22.32	86

Discussion

Considering the size of the animal and the distance covered, insect flight has to be considered as a very efficient way of progression in terms of the high metabolic activity and fuel consumption. The utilization of fat as a source of energy in insect flight is probably better understood than is generally realized for the majority of studies hitherto made, happened to have been concerned with insects which use carbohydrate as chief fuel (Chadwick, 1953). But in several insects, it is known or suspected that fat is largely utilized : *e g.* the desert locust (Weis-Fogh, 1952) Beet leafhopper (Fulton and Romney, 1940) Lepidoptera (Williams, 1945 ; Beall, 1948 ; Zebe, 1954, 57) dragon fly and bumble bee (George, Vallyathan and Scaria, 1958).

Our present observations on the flight muscles of some moths and the data obtained on the concentration of lipase in them suggest that moths too mobilize fat in their flight muscles for energy and that the lipase value could be regarded as an index of the measure of fat utilization. If this is accepted it is seen that moths are comparatively more active than butterflies. Among the butterflies studied by us (1960) the highest lipase value of 22.92 µl CO_2/mg protein/hr. was obtained for the migrant danaid butterfly, *Danais chrysippus* possessing the narrowest muscle fibres with an average diameter 80 µ and in the present study on some moths the two sphingid moths *Cephonodes hylas hylas* and *Polyptychus dentatus* were found to have the highest lipase value of 31.76 and 31.71 µl CO_2/mg protein/hr. respectively and having the narrowest fibres with a diameter of 75 µ. *Cephonodes hylas hylas* which is popularly known as the bumble bee hawk moth is a very swift and darting flier maintaining a steady degree of activity throughout the day while *Polyptychus dentatus* is also an active moth but nocturnal and readly attracted by light. The moths in general show a higher lipase value for their flight muscles than the butterflies. With the exception of two spiceis all the moths studied were the hawk moths belonging to the Family Sphingidae. The hawk moths in general with a few exceptions can be easily distinguished from other lepidopterans by their swiftness and body form in having long narrow pointed forewings, stout triangular hind wings, powerful thorax and sharply pointed abdomen. Several spicies of hawk moths are reported to be migrants (Williams, 1958) *viz. Hippotion celerino, Deilephila nerii, Herse convolvuli convolvuli* and *Acherontia styx styx* and related spicies. The minimum lipase value of 10.96 µl CO_2/mg protein/hr. obtained for *Acherontia styx styx* which is often included among the migrating hawk moths and was found to possess the largest muscle fibres with a diameter of 103 µ. In all the other hawk moths studied it was observed that with a decrease in the lipase value of the muscle there was a corresponding increase in the diameter of the respective muscle fibres (Table I). The two Noctuidae moths (*Nyctipao macrops* and *Ophideres meterna*) have an average diameter of 85µ and 86 µ and lipase values of 23.9 µl CO_2/mg protein/hr. and 22.3 µl CO_2/mg protein/hr. respectively, which as regards lipase values amount to more or less the highest figures obtained for the butterflies.

In classifying insect flight muscles on histological basis Tiegs (1955) included the lepidopteran flight muscles among the thinly fibrillated type which stands far below in evolutionary status than the coarsely fibrillated fibres of the higher insect orders such as Coleoptera, Hymenoptera and



Photomicrographs of transverse sections of the flight muscles of moths, treated histochemically to demonstrate the disposition of lipase.

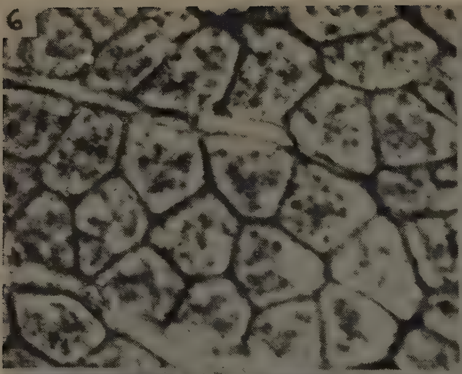
Fig. 1 *Cephonodes hylas hylas.* Note the high concentration of the precipitate.

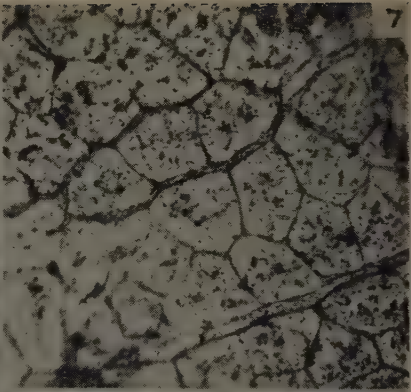
Fig. 2 *[illegible]* Note the equally high concentration of the precipitate as in Fig. 1

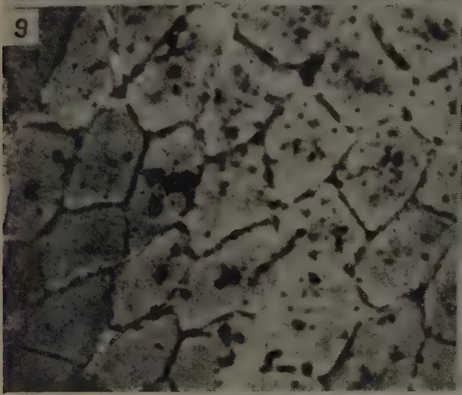
Fig. 3 *Hippotion rafflesi.*

Fig. 4 *Hippotion celerino.*

Fig. 5 *Deilephila nerii.*







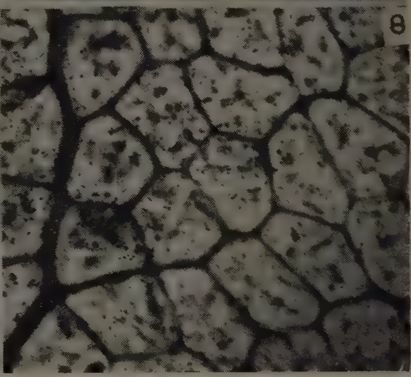


Fig. 6 *Nyctipao macrops.*

Fig. 7 *Ophideres meterna.*

Fig. 8 *Herse convolvuli convolvuli.*

Fig. 9 *Acherontia styx styx.* Note the low concentration of the precipitate and the large size of the fibres.

Diptera. He also observed that though the thrips and aphids are equipped with coarse sarcostyles in their flight muscle fibres, they are some of the feeblest fliers while of the butterflies with their thinly fibrillated fibres some are well known for their migratory flights. Stroboscopical examinations of the wing movements and the contractions of the flight muscles of some butterflies and moths (Tiegs, 1955) revealed that the moths have a greater wing beat frequency than the butterflies, showing thereby that the moths possess a more active flight musculature than the butterflies. These observations also lend support to the conclusion that the changes in the biochemical and physiological properties of a tissue are often accompanied by histological changes as well in its component cells and muscular tissue is not an exception to this.

The amount of lipase present in a muscle may be regarded as an indirect indication of the extent to which fat could be mobilized and utilized in that muscle. The data presented in this paper is further evidence in favour of such an inference. The comparatively higher concentration of lipase detected in the flight muscles of the moths studied, suggest the utilization of fat for energy during flight. Williams (1945) examined the fat content of two migrant moths and suggested that fat was the chief fuel for their migratory flight. Other experimental data (Williams, 1958) also show that a large number of the hawk moths indulge in migratory flights. An extensive study by Beall (1948) on the fat content of a butterfly (*Danais plexippus*) also is suggestive of the combustion of fat for energy. A study on the metabolism of some Lepidoptera (Zebe, 1954) showed that especially during flight, fat was used in the flight muscles. In the light of these observations it can be suggested that the amount of lipase activity in the flight muscles of the moths examined could be considered as an index of the extent of fat utilization in the muscle for energy.

The presence of the phosphatases (acid and alkaline phosphatases and ATPase) and succinic dehydrogenase in considerably large concentrations demonstrated by histochemical techniques shows that these muscles are metabolically very active. Although the precise physiological role of the alkaline and acid phosphatases is not so well understood as that of ATPase the former two have been shown to be important in the metabolism of lipids and carbohydrates and in the active transport of materials across a membrane barrier.

Summary

1 The fibre diameter of the flight muscles of a few moths was determined.

2 The following enzymes were histochemically detected in the flight muscles of the moths examined: lipase, acid and alkaline phosphatases, ATPase and succinic dehydrogenase. The distribution of the enzymes was found to be more or less uniform in the muscle fibres.

3 Quantitative determination of lipase revealed a marked difference in the various species studied. The results obtained provide further support to the view that moths also utilize fat for energy during muscular activity.

Acknowledgement

One of us (N.M.G.B.) is indebted to the Ministry of Education, Government of India, for the award of a Senior Research Scholarship which made this work possible. We are thankful to Mr. V. K. Chari, Curator of the Natural History Museum, Bombay, for helping us in identifying the moths used in our experiments.

References

Beall, G. 1948 The fat content of a butterfly, *Danais plexippus* as affected by migration. *Ecology*, **29**, 80-94.

Chadwick, L. E. 1953 The motion of wings. Aerodynamics and flight metabolism. The flight muscles and their control. In Roeder, K. D. Insect Physiology. New York

Edwards, G. A., Santos, P.deS., Santos H. L. deS and Sawaya, P. 1954a Electron microscope studies on insect muscle. I. The flight and coxal muscles of *Hydrophilus piceus*. *Ann. Ent. Soc. Amer.*, **47**, 343-54.

Edwards, G. A., Santos, P. deS., Santos, H. L. deS. and Sawaya. P. 1954 b Electron microscope studies on insect muscle. II. Flight and leg muscles of *Belostoma* and *Periplaneta*. *Ann Ent. Soc. Amer.*, **47**, 459-67.

Fulton, R. A. and Romney, V. E. 1940 The chloroform soluble components of beet leafhoppers as an indication of the distance they move in the Spring. *J. Agric. Res.*, **61**, 737-43.

George, J. C. and Bhakthan, N. M. G. 1960 A study on the fibre diameter and certain enzyme concentrations in the flight muscles of some butterflies. *J. Exp. Biol.*, **37**. (2), 308-315.

George, J. C. and Naik, R. M. 1958a The relative distribution and chemical nature of the fuel in the two types of the fibres in the *Pectoralis major* muscle of the pigeon. *Nature.*, **181**, 709-710.

George, J. C. and Naik, R. M. 1958b Relative distribution of mitochondria in the two types of fibres of the *Pectoralis major* muscle of the pigeon. *Nature.*, **181**, 782-83.

George, J. C. and Naik, R. M. 1959a The studies of the structure and physiology of the flight muscles of birds. 4. Fibre architecture of the *Pectoralis major* of the pigeon. *Biol. Bull.*, **116**, 239-48.

George, J. C. and Naik, R. M. 1959b Studies on the structure and physiology of the flight muscles of birds. 5. Some histochemical and cytochemical observations on the structure of the *Pectoralis*. *J. Anim. Morph. Physiol.*, **6** (1), 16-23.

George, J. C. and Scaria, K. S. 1956 On the occurrence of lipase in the skeletal muscles of of vertebrates and its possible significance in sustained muscular activity. *J. Anim. Morph. Physiol.*, **3** (2), 91-103.

George, J. C. and Scaria, K. S. 1958a Histochemical demonstration of lipase activity in the *Pectoralis major* muscle of the pigeon. *Nature.*, **181**, 783.

George, J. C. and Scaria, K. S. 1958b A histochemical study of succinic dehydrogenase activity in the *Pectoralis major* muscle of the pigeon and certain other vertebrate skeletal muscles. *Quart. J. Micro. Sci.*, **99** (4), 469-73.

George, J. C., Susheela, A. K. and Scaria, K. S. 1958 A quantitative and histochemical study of lipase in the *Pectoralis major* muscle of the bat. *Naturwissenchaften.*, **45**, 93-94.

George, J. C., Vallyathan, N. V. and Scaria, K. S. 1958 Lipase activity in the insect flight muscle. *Experientia.*, **14**, 250-51.

Gomori, G. 1953 Microscopic Histochemistry, Principles and Practice. University of Chicago Press.

Hawk, P. B., Oser, B. L. and Summerson, W. H. 1954 Practical Physiological Chemistry. McGraw-Hill Book Co. Inc.

Krogh, A. and Weis-Fogh, T. 1951 The respiratory exchange in desert locust (*Schistocerca gregaria*) before, during and after flight. *J. Exp. Biol.*, **28**, 344-57.

Pearse, E. A. G. 1954 Histochemistry, Theoratical and Applied. J. and A Churchill, London.

Tiegs, O. W. 1955 The flight muscles of insects—Their anatomy and histology with some observations on the structure of striated muscle in general. *Phil. Trans. Roy. Soc.*, **238**, 221-359.

Weis-Fogh, T. 1952 Fat combustion and metabolic rate of flying locusts (*Schistocerca gregaria* Forskal). *Phil. Trans. Roy. Soc.*, **237**, 1-36.

Williams, C. M. 1945 Notes on the fat content of two British migrant moths. *Proc. Roy. Ent. Soc.*, **30**, 6-13.

Williams, C. B. 1958 Insect Migration. Collins, London.

Zebe, E. 1954 On the metabolism of Lepidopters. *Zeit. Vergi. Physiol.* **36**, 290-317.

Zebe, E. 1957 Lactic and glycerophosphate dehydrogenase in insects. *J. Gen. Physiol.* **40**, 779-790.

[Received for publication on 15th April, 1960.]

EFFECT OF ESTRADIOL IMPLANTS ON THE GONADS AND OTHER ACCESSORY GENITAL ORGANS OF THE COMMON MALE TOAD, *BUFO MELANOSTICTUS* SCHNEID

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The effect of estrogen on males of various vertebrates has already been studied by several investigators. In mammals it is well known that the prolonged administration of estrogen to males produces atrophy of the testes and sex accessories (Golding & Ramirez, 1928; Emmens & Parkes, 1947; Turner, 1953; Williams, 1956). Our knowledge, however, regarding its effect on male Salientia is not adequate. Van Oordt and Klomp (1946) noted the distinct influence of oestrone only on the Müllerian duct of *Bufo bufo*. Cei *et al.* (1955) injected microcrystals of estradiol (aquous suspension) and noted specially the suppression of the spermatogenesis in *Leptodactylus chaquensis*.

Hermaphroditism is found in almost all the species of Bufo. The present purpose of our experiment is to observe the action of estrogenic hormone on the male and female components of the reproductive organisation in potentially male *Bufo melanostictus*. In Salientia oil solution has a damaging effect on the animals causing occlusion of the lymph heart (Houssay, 1954). In the present experiment we, therefore, used hypodermic pellets of pure crystalized estradiol and implanted them within the dorsal lymph sac.

Material and Methods

Common male toads (*Bufo melanostictus*) were collected locally prior to the onset of the experiment and were maintained under uniform husbandry conditions throughout the duration of the experimental period. Toads having a body length ranging from 67.0 to 75.0 mm. and body weight varying from 25 to 30 gms. were selected for our purpose.

The experiment was conducted in the month of August 1959 and was continued for two weeks. The animals were divided into the following groups :—

[J. Anim. Morph. Physiol., Vol. 7, No. 2, pp. 150-155, 1960]

Group A: *Normal unimplanted*—From freshly collected material five toads were selected for this group.

Group B: *Glass bead implanted*—A glass bead of about the same size and weight as the estradiol pellet was introduced into the dorsal lymph-sac of each of eight toads.

Group C: *Estradiol pellet implanted*—Ten toads were implanted with progynon pellets of 10 mg capacity one in each case through the dorsal lymph-sac.

The progynon pellets caused foreign body reaction as observed by Baker (1954) in rats, *i.e.* they became enveloped in a layer of connective tissue, in which the blood capillaries developed. The glass bead did not induce any such reaction. From the remnant of the Progynon pellets of experimental animals the fibrous connective envelopes were removed, dried and weighed. It appeared that approximately 2/3 mgs were absorbed in most cases.

Following autopsy the testis, Bidder's organ and the Müllerian duct were fixed in Bouin's fluid for histological studies. Seven micron thick sections were taken and stained with haemalum—eosin. Along with other observations the epithelial height of the Müllerian duct, the diameter of the seminiferous tubules and the Bidder oocytes were measured.

Observations

Testis:

Control (Group A & B)—The relative testicular weight in relation to the body weight of experimental individuals at the time of autopsy was more or less same. Sections through the testis show the presence of primary spermatogonia both in resting and divisional stages. Cell nests containing secondary spermatogonia are very few and in some tubules they are completely absent. Spermatocytic cell nest is very predominant in most of the testis follicular components. Sperm bundles are also found embedded in the sertoli elements within the tubular lumen.

In general interstitial cells are not very well developed in toads. However, the individuals of this group show the usual normal picture.

Treated (Group C)—A marked decrease of about 0.5% of the relative testis weight is observed in these animals. The cellular picture of the seminiferous epithelium is quite constrasting in comparison with the above control groups. In all individuals of this group spermatogonial and spermatocytic stages are practically absent. In a very few tubules the above mentioned cell nests are sporadically present and exhibits a definite disorganisation in their cellular components. But spermatids and sperm bundles are very abanduntly found throughout the tubules in all the individuals.

The interstitial cells also undergo some disorganisation. The loss of the plasma membrane accompanied by a vacuolation of cytoplasm and shrinkage of the nucleus is observed. The eosinophil colloids are found to accumulate in the tubular space.

Bidder's Organ :

Control (Group A & B)—Fig. 1.—The Bidder oocytes of this group appear to resemble the normal ovarian picture. They are represented in various stages of development. Generally the smaller oocytes are situated on the periphery with less amount of yolk. The nuclear membrane is also not very well demarkated from the cytoplasm. In bigger oocytes the nucleus is situated in the centre and the yolk matter is coarse and granulated. The diameter of the ova is distinctly larger than the treated oocytes. Some pre-ovulatory corpus luteum has also been observed in both the groups.

Treated (Group C)—Fig. 2.—Macroscopically the Bidder's organ shows an increased pigmentation all over the external surface. The diameter of the oocytes decrease considerably in comparison to the controls. In most cases the nuclear membrane disappears and a cavity is formed inside the oocytes. The " liquefaction " of the oocytes are also observed. Eiosinophilic nature of the nuclear material considerably increases. But the structure of the corpus luteum is similar to that of controls.

Mullerian Duct :

Control (Group A & B)—Fig. 5. The Müllerian ducts of the groups of animals are very thin, undifferentiated and pigmented cord attached to the peritoneal membrane and extend from the cloacal region to the oesophageal level. Microscopic structure of the Müllerian duct reveals that its lumen is very narrow, with the absence of villi in the mucosal layer. The muscular layer is extremely thin and is represented by a few layer of muscle fibres.

Treated (Group C)—Fig. 3 & 4. This duct becomes a much convoluted and whitish cord-like structure after treatment. The portion of

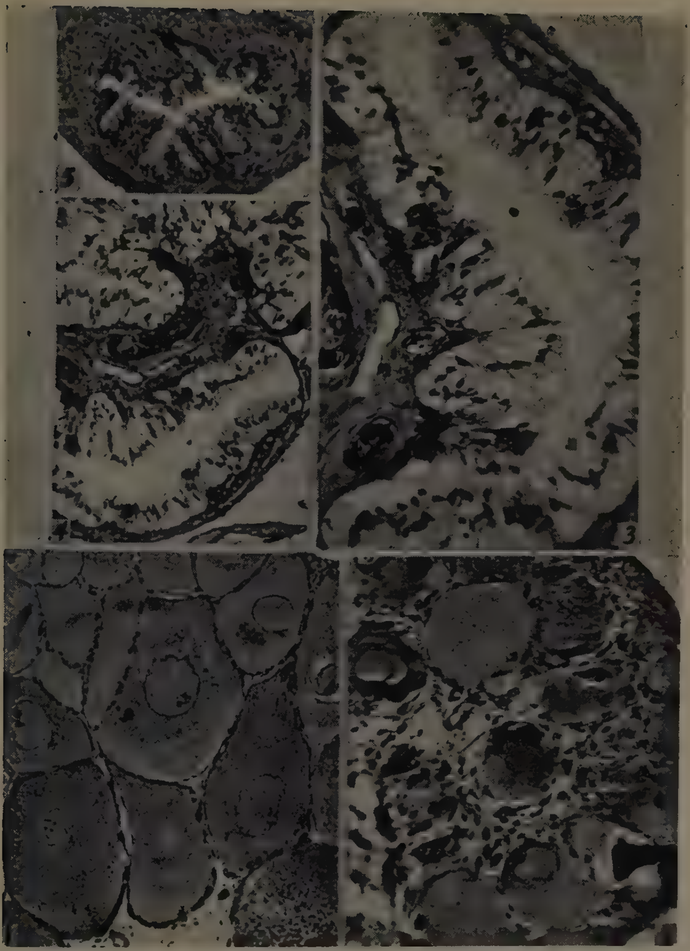

Plate I

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Fig. 1 Section (7 μ) through the Bidder's organ of a glass-bead implanted control toad showing the Bidder oocytes of different stages of development. (X 325).

Fig. 2 Section (7 μ) through the Bidder's organ of Progynon pellet implanted toads. Note the disorganisation and liquifaction of the Bidder oocytes. (X 325).

Fig. 3 Magnified view of the Müllerian duct of Progynon pellet implanted toad. Note the epithelial height and the mucous secreting glands. (X 580).

Fig. 4 Section (7 μ) through the convoluted portion of the Müllerian duct of Progynon pellet implanted toad. Note the pronounced convolutions and the enlarged lumen inside. (X 325).

Fig. 5 Section (7 μ) through the Müllerian duct of a glass bead implanted control toad, showing the narrow lumen and the absence of villosity in the mucous layer. (X 325).

the duct near the lower end of the kidney develops some convolutions, but takes a tortuous path and traverses upwards above the end of the kidney and gradually becomes narrower. The histoarchitecture of the duct shows a pronounced enlargement of the lumen, especially in the convoluted region. The mucosal layer develops considerably forming numerous villi inside the lumen. The highest region of the epithelial layer measures approximately 1.78 microns. The mucous secreting glands are also very well developed. Thickening of the surrounding muscle layer is also observed.

Discussion

Exogenous estrogen acts as a spermatogenetic inhibitor in males (Emmens and Parkes, 1947). Moore and Price (1930) experimentally proved that the effect of the estrogen is exerted by depressing the gonadotrophic factor of the pituitary. Later, this opinion was supported by several investigators in this field. Cei *et al* (1955) reported the suppressive effect of estrogen (microcrystals of Progynon-Schering) on the spermatogenesis of *Leptodactylus chaquensis*. From our present observations we are inclined to corroborate the findings of previous investigators. But curiously enough in *Bufo melanostictus* the testis of treated individuals show the presence of higher spermatogenetic stages and the spermatogonial cells are absent. This indicates the blocking effect of further division on the initial stages of spermatogenesis. It further proves that prolonged treatment will produce a complete degeneration of the testicular follicles either by phagocytosis or disintegration of the spermatids and sperm bundles which is predominant in the present observation. The cellular disorganisation of the male gonad indicates the absence of FSH secretion from the pituitary and interstitial cell components further prove the lowering of LH factors.

Bidder's organ in toad is a non-functional ovary. Pituitary plays a definite role in the maintenance of the Bidder's organ as has already been proved experimentally by De Groot *et al* (1948) in *Bufo bufo*. They observed that hypophysectomy causes the degeneration of the Bidder's organ mainly by "liquefaction" of the Bidder oocytes. On the other hand the experiment of Ponse (1927) demonstrated that removal of testis causes the growth of Bidder's organ just due to the absence of the inhibitory effect of the testicular hormone. It is therefore, expected that estrogen will enhance its growth. But the observation of our present experiment proves that in toads the opposite action takes place. This may be due to over dosage of estrogen treatment. But the quantity of absorption is quite normal and seems to cause no abnormality due to superactivity. So far as the path of action is concerned it is of interest to note that the testis atrophy proves that estrogen acts through the pituitary, depressing the gonadotrophic factors. However, it is clear that the blocking of pituitary gonadotrophin is further responsible for the atrophy of the Bidder's organ as observed in cellular components of the testis follicles of *Bufo melanostictus*.

Van Oordt and Klomp (1946) noted that oestrone injected male toads has a distinct influence on the Müllerian duct of *Bufo bufo* when injected during summer months. Our present experiment also shows a similar effect on the Müllerian duct. This indicates that estrogen, particularly in the case of the Müllerian duct has acted directly without intermediation of pituitary. This activated picture of the Müllerian duct indicates that the absorbed estrogen was not wasted and that the abnormal pictures of testis and Bidder cellular components were mainly due to the suppressive effect of estrogen mediated through the pituitary.

In this experiment it is clear that estradiol pellets when absorbed acts through pituitary. Consequently, we can except the cellular changes in the adenohypophysis of treated toads with special reference to gonadotrophs. This may either cause cellular atrophy or the arrest of further secretary function of the cellular components. At persent we are studying pituitary cell changes following Progynon implantation.

Summary

1 Estrogen acts as an inhibitory substance on both testis and Bidder's organ in *Bufo melahostictus*. This is due to the blocking effect of estrogen on Pituitary gonadotrophin secreting cells or an atrophy of the same.

2 Müllerian duct is very well developed in treated individuals. It is also proved that absorbed estrogen acts directly on this underdeveloped oviduct of male toads.

3 The well developed Müllerian duct of treated individuals further indicates that absorbed estrogen acted properly and the inhibitory effect on the testis and Bidder cellular components is not a false reaction.

4 Possible significance of the action and interaction of estrogen and its relation with pituitary and the terget organs are discussed.

Acknowledgment

We express our sincere thanks to Prof. J. L. Bhaduri, Head of the department of Zoölogy, Calcutta University, for his constructive suggestions and encouragements. We are deeply indebted to Dr. A. Ghosh, Histophysiology laboratory, Calcutta University, for going through the manuscript.

We also express our sincere gratitude to the director, Schering Asia, Berlin, for sending us a gratuitous gift of Progynon implants for our experiments. The photomicrographs were taken by Mr. M. N. Dutta to whom our thanks are due.

Reference

Baker, B. L., 1954. The connective tissue reaction around implanted pellets of steroid hormones. *Anat. Recd.*, **119**: 4; 529.

Cei, J. M., Andreozzi, M. L. and Acosta, D. L., 1955. Hormonas esterolicas y correlaciones hipofiso—genitales. *Arch. Farm. Bioquim*, **7**: 119.

De Groot, A. A., de vries, G. A. and Mighorst, J. C. A. 1948. Effect of hypophysectomy on the male toad (*Bufo bufo* L.) with special reference to Bidder's organ. *Proc. Konik. Neder. Akad. Wetenschap.*, **51**: 121.

Emmens, C. W. and Parkes, A. S., 1947. Effects of exogenous estrogens on the male mammals. Vitamins and Hormones, New York, Academic Press Inc., **5**: 233.

Golding, G. T. and Ramirez, F. T, 1928. Ovarian and placental hormonal effects in normal, immature Albino rats. *Endocrinol*, **12**: 804.

Moore, C. R. and Price, D. 1930. The question of sex hormone antagonismi *Proc. Soc. Expt. Biol. Med.*, **28**: 38.

Ponse, K., 1927, L'evolution de l'organe de Bidder et la sexulite chez le crapaud. *Rev. Suisse Zool.* **34**: 217.

Turner, C. D., 1953, General Endocrinology. W. B. Saunders company, London.

Van Oordt, G. J. and Klomp, H., 1946, Effect of oestrone and gonadotrophin administration in the male toads (*Bufo bufo*). *Proc. Konik. Neder. Akad. Wetenschap.*, **49**: 565.

Willams, R. H., 1956. Text Book of Endocrinilogy. Saunders and Co. Philadelphia.

[Received for publication on 8th April, 1960]

THE DIGESTIVE ENZYMES OF THE TERMITE *HETEROTERMIS INDICOLA*

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Termites make use of the products of cellulose after its digestion by symbiotic protozoan fauna of their gut (Trager, 1932). There is evidence that if the fauna is removed by some method, defaunated animals failed to digest cellulose (Cleveland, 1924). There is evidence also to show that the bacteria and spirochaetes occurring in the gut, are not responsible for cellulose digestion (Cleveland, 1926). Wood eaten by termites contains 54.6% cellulose, whereas their faecal cellulose is only 18% (Wigglesworth, 1953). In addition to cellulose, wood also possesses 2 to 14% hexosans; 0 to 5.9% starch; 0 to 6.2% other sugars and 1.1 to 2.3% proteins. Of course, termites do not live solely on cellulose even though there is evidence that they utilize 2/3rd of the ingested cellulose. Now it seems to be correct that neither the host nor its symbonts are able to fix atmospheric nitrogen (Tóth, 1946). Also, it is already known that the termites do not mature or reproduce solely on pure cellulose without nitrogen (Hungate, 1943). They require some nitrogen of outside origin which is essential for maturation and reproduction. Therefore it has long been predicted that there are some proteolytic enzymes in termites which essentially digest the protein nitrogen and supply it to the organism. It is conceivable that the nitrogen from the ingested wood, would be sufficient for the organism. To digest the little quantity of protein in the wood and thus get the required nitrogen the termites should possess proteolytic enzymes.

There is some information regarding the digestive enzymes present in termites which gives us an indication of what constituents of wood other than cellulose are used by the termites. As stated above wood contains 0 to 5.9% starch. An amylase has been found in the midgut of *Calotermis* (Visintin, 1947). In the foregut of *Zootermopsis* was found an amylase, and a proteinase was found in the midgut (Hungate, 1938). Similarly it would be clear from the feeding habits of the termites that they must be possessing enzymes for the digestion of fats and sugars. In support of this statement, termites are found to be feeding not only on dried bark and logs of wood but also on dried sugarcane plants (a rich source of sugars) and sometimes on seedlings rich in oils. So a detailed investigation of the enzymes for digesting the protein, sugar, fats and oils of wood on which the termite is feeding would be of interest.

Materials and Methods

Specimens of *Heterotermis indicola*, the common South Indian termite, were used for the investigation. Since the individuals were small in size, crude extracts of whole animals in a considerable number were used for the purpose. These are prepared by grinding a number of termites in a mortar with 5 cc of distilled water. The homogenate was centrifuged and the resulting solution was used in the enzyme assays. Only fresh extracts were used for the investigation.

For quantitative estimations, the results obtained in the experiments by using these extracts were divided by the number of termites used for the preparation of the same. Thus the average activity of the enzyme in one individual is obtained in every case.

The protozoan defaunation methods were followed according to Cleveland (1926). The three main methods were used *viz.* incubating the termites in humid air at about 36° to 38°C for 24 hours ; by putting the termites in an atmosphere of pure oxygen for 6 days and thirdly, starving the specimens without any kind of food at room temperature for 10 days.

Substrates and Tests for enzyme detection

For invertase, amylase and lipase qualitative tests, as given by Welsh and Smith (1953) were followed. Sucrase, maltase and lactase were studied according to methods described in Hawk, Oser and Summerson (1954).

For qualitative estimations of peptic ferments, Trypsin and Chymosin, the methods offered by Pavlovsky and Zarin (1926) were followed.

Cathepsin was estimated by the time taken for the liquifaction of 10% gelation at a pH of 4, since the optimum pH of cathepsin is usually around 4. For qualitative measurements of peptic ferments, the procedure given by Volhard and Lohlein was followed as described in Hawk, Oser and Summerson (1954).

For qualitative estimations of rennin and amylase methods given in Hawk, Oser and Summerson (1954) were followed.

Estimation of lipase was done according to the procedure found in Welsh and Smith (1953). For the estimation of chymosin, the method given by Pavlovsky and Zarin (1926) was followed.

Results

The results of the study, showing the occurrence of the various digestive enzymes in the differently treated individuals of *Heterotermis indicola* are summarized in the accompanying table.

Tabulated statement of the enzymes present in the gut of Heterotermis indicola

Enzyme	Freshly collected termites	Termites starved for 10 days	Termites exposed to pure oxygen for 6 days	Termites incubated at 37°C for 2 days
Proteolytic enzymes				
1 Peptic ferments	0.324 units	0.0683 units	0.648 units	0·144 units
2 Trypsin	Absent	Absent	Absent	Absent
3 Cathepsin (activity is measured for complete digestion of the substrate given)	3·75 minutes	3·75 minutes	9 minutes	9 minutes
4 Chymosin (time for coagulation of milk)	1.75 minutes	0.467 minutes	.4 minutes	1.83 minutes
5 Rennin	0.275 units	0.066 units	0.65 units	0.322 units

Discussion

It is known that termites possess a symbiotic fauna which are responsible for cellulose digestion (Cleveland, 1924). The results presented above show that the termites possess also proteolytic enzymes like the peptic ferments cathepsin, chymosin and rennin; lipolytic enzymes like lipase, and carbo-hydrases like amylase, sucrase maltase and lactase. Most of these enzymes appear to be produced by the termites themselves and are not derived from the symbionts.

Tabulated statement of the enzymes present in the gut of Heterotermis indicola (continued)

Enzyme	Freshly collected termites	Termites starved for 10 days	Termites exposed to pure oxygen for 6 days	Termites incubated at 37°C for 2 days
Carbohydrases				
1 Amylase	0.33 mgs. of starch	0.32 mgs.	0.4 mgs.	0.475 mgs.
2 Invertase	+	+	+	+
3 Sucrase	+	+	+	+
4 Maltase	+	+	+	—
5 Lactase	+	+	+	—
Lipolytic Enzymes				
1 Lipase activity after 1½ hours	0.01 cc	0.01 cc	0.0083 cc	0.00857 cc

The absence of trypsin either in the defaunated or fresh specimens of termites looks somewhat curious, deviating from the normal rule. Generally the invertebrates possess the proteolytic enzymes of the tryptic type. The possession of proteolytic enzymes of the tryptic type is considered to be characteristic of insects (Wigglesworth, 1953). The termites, on the contrary, seem to possess proteolytic enzymes of the peptic type such as chymosin, cathepsin and rennin. This apparent anomaly can be explained on the basis of acid nature of the gut as shown by Hungate (1943), who explains the acidity in the alimentary canal of termite, as being due to the formation of acetic acid as a fermentation product. The protozoan fauna convert cellulose into sugars which are then fermented by bacteria in the gut. The acetic acid thus formed is absorbed in the hind gut of the termite (Hungate, 1943).

Cathepsin activity in fresh specimens is greater than in the defaunated specimens. In fresh specimens cathepsin takes less time to liquify or digest the gelatin, whereas in the defaunted specimens it takes a longer time. According to Krijgsmann (1936) the protozoa contain proteolytic enzymes like cathepsin. Fresh specimens contain protozoans which have cathepsin. The defaunated specimens lack protozoans and hence there is a decrease in the cathepsin activity in the defaunated termites.

The amylase and lipase activities are almost the same before and after defaunation. It would mean that they are not affected by the removal of the intestinal fauna. Therefore these enzymes are principally produced by the host gut itself and do not appear to belong to the protozoan fauna. The situation seems to be similar with regard to invertase, sucrase, maltase and lactase.

When the anterior region of the gut *i.e.*, the region upto and inclusive of the gizzard was seperated and its contents observed under the phase contrast microscope, a very rich protozoan fauna was observed. But the part of the gut posterior to the gizzard was very poor in protozoan fauna but very rich in bacteria. This would speak of the possible occurrence of the proteolytic enzymes in the posterior region of the gut since their presence in the anterior region would have been detrimental to the protozoan fauna. Hungate's (1938) finding of a proteinase in the midgut of *Calotermis* also adds some weight to the above conclusion. As the food passes from anterior region to the posterior region in the gut, some of the protozoans are carried into the posterior region and are there digested by the enzymes. But the fauna in the anterior part of the gut would continue to thrive and multiply.

Summary

Fresh as well as defaunated termites possess proteolytic enzymes such as chymosin, cathepsin and rennin; such carbohydrases as amylase, invertase, sucrase, maltase and lactase, and also a lipase. The fact that there is no significant difference in the enzyme activity in the defaunated as compared to fresh termites, indicates that these enzymes are produced by the termite gut and not contained in or produced by the symbiotic fauna.

Trypsin is singularly absent. Cathepsin appears to be a product of the intestinal fauna.

In view of the large population of the symbiotic protozoa in the anterior part of the gut, while the posterior part of the gut is nearly free of the protozoans, it is possible that that part of the protozoan fauna which happens to get into the posterior part of the gut is digested and utilized.

Acknowledgment

My sincere gratitude to Prof. Kandula Pampapathi Rao, Head of the Department of Zoology for suggesting this problem for investigation, for giving inspiring suggestions whenever needed and finally examining the paper critically.

References

Cleveland, L. R. 1924. Symbiotic relationships of Protozoa and Termites. *Biol. Bull.* **46**: 177-225.

Cleveland, L. R. 1926. Symbiosis among Animals with special reference to termites and their intestinal flagellates. *Quart. Rev. Biol.* **1**: 51-60.

Hawk, P. B., Oser, B. L. and Summerson, W. H. 1954. Practical Physiological chemistry McGraw-Hill Book Company, Inc. New York.

Hungate, R. E. 1938. Wood digestion, Protozoa and Termites. *Ecology*, **19**: 1-25.

Hungate, R. E. 1943: Cellulose Fermentation in Termite Protozoa. *Ann. Ent. Soc. Amer.* **34**: 467-489.

Krijgsmann 1936. *Zeitchr. Vergl. Physiol.* **23**: 663. (Quoted by Heilbrunn in his General Physiology).

Pavlovsky and Zarin. 1926. On the structure and ferments of the digestive organs of scorpion. *Quart. J. Micro. Sci.* **66**: 509-556.

Roeder, K. D. (Ed.) 1953 Insect Physiology. Chapman and Hall Ltd., London.

Toth, L. 1946 Biological fixation of atmospheric Nitrogen. *Hungarian Museum of Nat. Sciences*, 116 pp. Budapest (Quoted from Roeder).

Trager, W. 1932 Cellulose from intestinal flagellates of *Cryptocercus* (Blattidae). *Biochem. J.* **26**: 1762-71.

Visintin, B. 1947 Starch nutrition of *Calotermis*. Rend. Ist Super Sauita 10: 290-300. (Quoted from Roeder).

Welsh, J. H. and Smith R. J. 1953 Laboratory exercises in Invertebrate Physiology. Burgess Publishing Company, Minneapolis.

Wigglesworth, V. B. 1953 The principles of insect Physiology. London, Methuen & Company Limited.

[Received for publication on 23rd May, 1960]

STUDIES ON ASCORBIC ACID IN FISH TISSUES. I.

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VERY few studies have been made on ascorbic acid in fish tissues. Love (1958) cites about ten references relating to the ascorbic acid content in fish tissues, most of which are not recent. Data from analyses on preserved fish, or "purchased" fish cannot be considered as reliable. An instance of this, referred to by Love (1957), is the conclusion of Chatfield (1954) that vitamin C content of fish tissues is negligible, whereas actually appreciable quantities may be present in certain tissues.

In the investigation of ascorbic acid in tissues, an aspect which deserves mention is the realization of its existence in the free and bound states. It is now known that most of the ascorbic acid in tissues is free, while the rest which is bound, is released only by hydrolysis (Haurowitz 1959). The bound form known as ascorbigen seems to be a condensation product of ascorbic acid with indole derivatives (Haurowitz 1959). Hastings and Spencer (1952) determined free and bound ascorbic acid in Albacole, Silver Salmon, Pacific Herring and remarked that the value of determining free and bound ascorbic acid is not understood.

The present authors have been investigating the ascorbic acid content of fish tissues. In some of these, like the gonad and brain, quite significant quantities are present. The present account, however, is confined to the ascorbic acid content of the integument and muscle of a few estuarine and marine fishes. The estimation of ascorbic acid in the skin was undertaken as a part of our study of the composition and physiology of the skin of fishes, as a preliminary step to the understanding of any possible relationship between vitamin C and morphogenesis of scales and scale regeneration. With regard to the vitamin C content of muscle, besides the determination of total ascorbic acid by the usual method employing cold extraction with TCA (Thichloro-accetic acid), free and bound moieties of the ascorbic acid were determined in a few samples. This was chiefly to find out whether the total levels obtained by the two methods are considerably different.

[J. Anim. Morph. Physiol., Vol. 7, No. 2, pp. 162-165, 1960]

Material

All the specimens investigated were obtained in the fresh condition for vitamin C content in the skin, the following fish were investigated :—

1. *Mugil cephalus* Linn. 2. *Liza dussumeirii* (Val). 3. *Boleophthalmas boddaertii* Pallas. 4. *Tochysurus venosus* (Val) and 5. *Ophichthys boro* (Ham. Buch). The vitamin C content of muscle was studied in 1. *Mugil cephalus*, 2. *Liza dussumeirii*, 3. *Boleophthalmus boddaertii*, 4. *Tachysurus venosus* and 5. *Tilapia mossambica*. (Peters)

Free and bound ascorbic acid contents were determined in the muscle of *Liza dussumeirii* and *Tilapia mossambica*. The effect of deep freezing for 72 hours on the vitamin C content of muscle in two species was also studied.

Analytical Procedure

Ascorbic acid determinations were made adopting the method of Roe and Keuther as described by Hawk *et al.* (1954). For the determination of free and bound fractions of ascorbic acid, the procedure of Summerwell and Sealock (1951) was followed, using ice-cold ethanol for extraction of free ascorbic acid, and hydrolysing with TCA the residue after ethanol extraction for liberation of the bound form. Colorimetric estimations were carried out using a Klett-Summerson photoelectric Colorimeter with filter no. 54.

Results and Discussion

The results of analysis are shown in Tables I to IV.

I. *Ascorbic acid content in skin*

	*mg per 100 gm.	Range
Mugil cephalus	2.26	1.31 to 3.2
Liza dussumierii	2.21	1.3 to 3.0
Boleopthalmus boddaertii	2.19	1.66 to 2.85
Tachysurus venosus (*Anius malabaricus*)	0.784	0.22 to 1.43
Ophichthys boro	0.085	0.05 to 0.11

* Mean values for eight determinations.

II. Ascorbic acid content in muscle

	*mg per 100 gm.	Range
Mugil cephalus	1.2	0.71 to 1.29
Liza dussumierii	1.07	0.73 to 1.25
Boleopthalmus boddaertii	0.512	0.23 to 0.7
Tilapia mossambica	0.95	0.84 to 1.07
Tachysurus venosus	1.12	0.36 to 2.3

* Mean values for eight determinations.

III. *Comparison of ascorbic acid values obtained by determinations of free and bound fractions with the value obtained by single TCA extraction*

	Free	*Bound*	*Total*	*Single TCA extraction*
Liza dussumierii	0.773	0.35	1.123	1.25
Tilapia mossambica	1.029	0.252	1.281	1.309

Mean values for four determinations expressed as mg. per 100 gm. of tissue.

IV. *Effect of deep freezing on vitamin C content in muscle in mg. per 100 gm. of tissue*

	0 hours	*24 hours*	*48 hours*	*72 hours*
Liza dussumierii	1.12	1.10	1.10	1.08
Tachysurus venosus	0.86	0.857	0.857	0.854

From Table 1, it will be observed that the mean values for the ascorbic acid content of the skin of the " scaleless fish " is lower than that of the scaly fish. Robertson (1953) has suggested that ascorbic acid may be of importance for the formation of the prolines of collagen in healing wounds. Gustavson (1956) has suggested that ascorbic acid may be necessary for hydroxyproline, the characteristic amino acid constituent of collagen. It is not possible to state without further investigation how the low ascorbic acid content of the skin and absence of scales in *Ophichthys* and in the catfish, *Tachysurus* are related.

The vitamin C content of muscle in the fishes investigated, is not much. It is more or less comparable to that of Silver Salmon investigated by Hastings and Spencer (1952).

From table 3, it could be seen that the total levels of ascorbic acid obtained by adding free and bound ascorbic acid fractions are not significantly different from those obtained by a single cold extraction with trichloroacetic acid.

The effect of deep freezing on vitamin C content of muscle is shown in table IV. This was studied for only 72 hours, but even during this period, there was slight reduction.

Summary

1. The vitamin C content of the integument and muscle in five fishes has been investigated.

2. The vitamin C content in the skin of scaleless fishes *Ophichthys boro* and the catfish, *Tachysurus venosus* is lower than that in the skin of the scaly fishes investigated.

3. The total ascorbic acid content in muscle obtained by adding the free and bound fractions, is not significantly different from that obtained by single cold extraction with TCA.

4. Ascorbic acid content diminishes in frozen muscle.

Acknowledgement

Grateful acknowledgement is made to Prof. R. V. Seshaiya, Director, Marine Biological Station, Porto Novo. for instruction and guidance and to the University Grants Commission for the award of Research Scholarships to both of us.

References

Chatfield, C. 1954 "Food Composition Tables"—Minerals and Vitamins, p. 44. F.A.O., Rome.

Gustavson, K. H. 1956 The Chemistry and Reactivity of Collagen, 78. Academic Press Inc. New York.

Hastings, W. H. & Spencer, C. F. 1952 Determinations of Free and bound Ascorbic acid in fishery products. *J. Mar. Res.* **11**, 241.

Haurowitz, F. 1959 Progress in Biochemistry since 1949, 118 Interscience Publishers, Inc. New York.

Hawk, P. B. *et. al.* 1954 Practical physiologica chemistry, 1236.

Love. R. M. 1957 Biochemical composition of fish. The physiology of fishes, Ed., Brown, M. B.,–1. 401. Academic Press Inc. New York.

Love, R. M.:, Lovern, I.A. & Jones, N. R. 1959 The Chemical Composition of Fish Tissues., B. Department of Scientific and Industrial Research: Food Investigation Special Report. no. 69. H.M.S.O. London.

Robertson, W. Van B., & Schwartz B. 1953 Ascorbic acid and the formation of collegen. *J. Biol. Chem.* **201**, 689.

Roe, J. H. 1959 Chemical determination of Ascorbic, Dehydroascorbic and Diketogulonic acids. Methods of Biochemical Analysis, Ed. Glick, D.–**1**, 127. Inter Science Publishers, Inc. New York.

Summerwell, W. N. & Sealock, R. R. 1951 Bound ascorbic acid in Liver Tissue. *J. Biol. Chem.* **196**, 753.

[Received for publication on 29th October, 1960]

SHORT COMMUNICATIONS

AN INTERESTING ANTENNO-FRONTAL STRIDULATORY MECHANISM IN A GRYLLID, *LOXOBLEMMUS EQUESTRIS* SAUSSURE (ORTHOPTERA)

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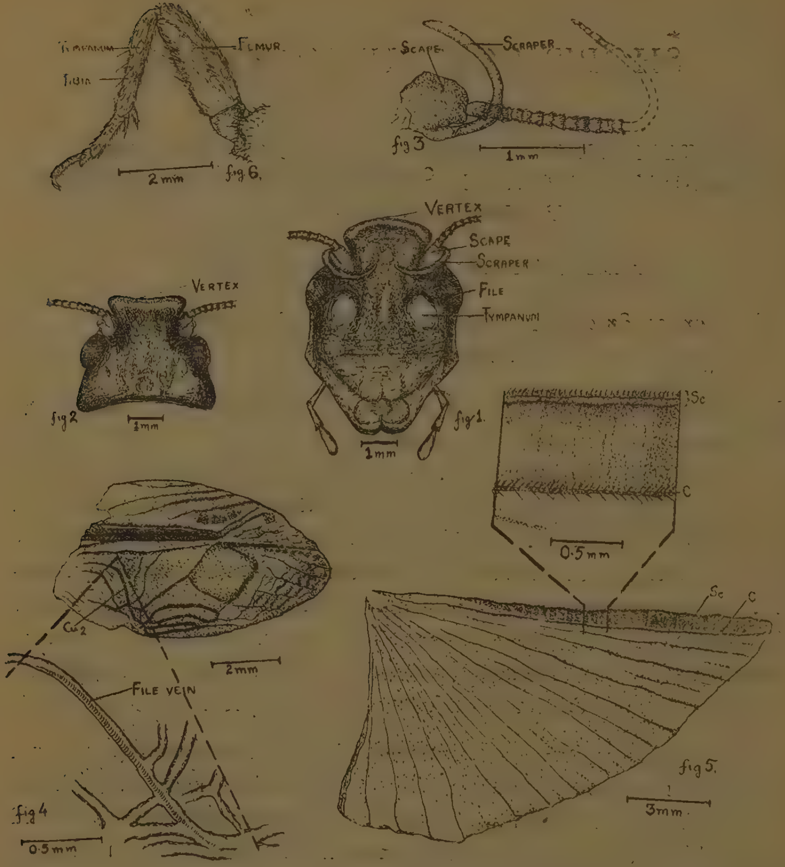
DURING the course of general collection of insects in Delhi one of the authors came across with a peculiar gryllid attracted to light during the monsoon days. This species has since been successively collected for three years during the same period and under the same circumstances and same locality. Reference to the National Pusa Collection showed that the species in question is *Loxoblemmus equestris* Saussure and some specimens from Pusa, Bihar, were also observed in this collection. The peculiarity of this gryllid consists of the presence of an antenno-frontal stridulatory apparatus present on the head of the males. The females, of the species, in the National Pusa Collection do not present this peculiarity.

The stridulatory mechanism mentioned above consists of an antennal hook and a frontal ridge in front of the antennal sockets on which the hook could be scrapped and an amplyfying tympanal area in front of and adjoining this ridge (fig. 1). The antennal hook borne on the broad basal scape of the antennae, is highly chitinised, cylindrical, curved inwards, and blunt at the tip (fig. 3). This hook forms the 'scrapper'. The tympanal areas for the amplification of the sound produced lie on either side of the frontal area in front of the antennal sockets and mesad of the compound eyes. Each is obliquely oval in shape and its marginal half, adjoining the antennae and eyes, is thickened and well sclerotised to form the ridge which may be considered as the 'file'. Under higher magnifications this is really found to be composed of a series of concentric ridges. The tips of the scrapper hooks are rubbed against the file ridges for the production of sound as the antennae are waved to and fro. The fact that this apparatus

[J. Anim. Morph. Physiol., Vol. 7, No. 2, pp. 167-169, 1960]



Loxoblemmus equestris Saussure : Fig. 1. Frontal view of *Head* ; Fig. 2. Dorsal view of *Head* ; Fig. 3. *Antenna* ; Fig. 4. Tegmina with *File vein* magnified ; Fig. 5. Hind wing with *Costal* margin magnified ; Fig. 6. *Fore-leg.*

is not found in the females lends support to the view that the sound produced by this mechanism may be considered as sexual calls of the males.

Despite the presence of the antenno-frontal stridulatory apparatus mentioned above, it is interesting to note, that the normal gryllid type of tegminal stridulatory structures too are present in this species. The tegminae (fig. 4) have the second cubital vein inwardly thickened, highly arched and with a series of peg-like prominences to form the file, but the scrapper and amplyfying tympanal areas on them appear to be rather poorly developed. It is also noteworthy that along the costal cell of the hind wings more than half the wing-length distally is occupied by a series of parallel cross-veins (fig. 5); perhaps these also take part in stridulation functioning as a scrapper.

The males of *Loxoblemmus equestris* can be readily distinguished from the females and also from other gryllids by their broad truncated vertex of the head (fig. 2). Further, the frontal area in them is rather flat or even slightly concave as against the convex frontal area of others.

The auditory mechanism in these gryllids consists of the usual tympanal areas borne on the bases of the fore tibiae (fig. 6).

[Received for Publication on 12th June, 1960].

A DEVICE FOR MEASURING THE OXYGEN CONSUMPTION OF SMALL ORGANISMS

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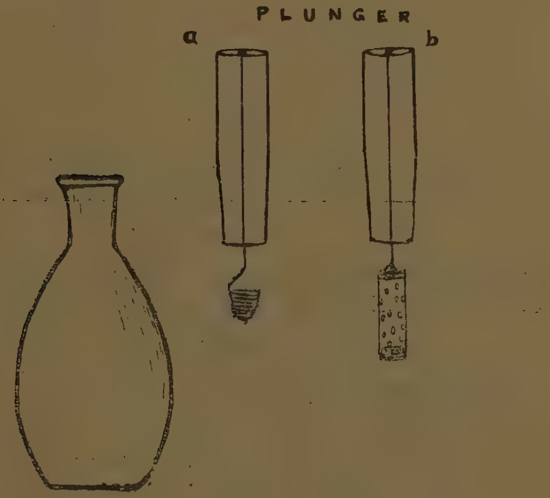
IN the course of the study of metabolism in fish with reference to age and size and under different conditions of dissolved oxygen, temperature, pH and salinity, the apparatus used by the author and described in Job 1957, was found to be efficient as long as the size of the subject in question was above 0.5g and volume of the water was over 75 ml in the respiration chamber. The apparatus described here was therefore developed with the object of extending the size range from the egg stage to the post-larva in the experiments. In this laboratory it has now been successfully adopted for similar studies on other small organisms such as an amphipod.

Briefly, the original apparatus used was just a 5 ml specific gravity bottle. Through the bore of the stopper a fine stainless steel wire is passed and is sealed with paraffin wax. The free end of the wire is either coiled into a basket or holds attached to it, a piece of perforated polythene tubing open at the free end. (Fig. 1). Eggs and non-motile pro-larva could be placed in the basket (a) and the motile forms enclosed in the polythene jacket, (b) and the open end of the latter closed. The stopper carrying these is called the plunger. Water of known quality is filled in two similar bottles, one being used as the respiration chamber and the other as the control. After placing the specimen in the basket or the jacket the plunger is quickly introduced into the chamber. When it is time for the first reading to be taken the plunger together with the specimen is quickly transferred to a second respiration chamber with very little or no water from the first and the experiment continued. Almost simultaneously the Winkler reagents for oxygen suitably reduced to the volume of water (5 ml) to be tested, are introduced into the first chamber and is closed with a spare stopper. The entire sample in the chamber is now analysed for oxygen.

Objections may be raised in regard to the time lag involved in

[J. Anim. Morph. Physiol., Vol. 7, No. 2, pp. 170-172, 1960]

transfers from one chamber to the next and the exposure of the animal in that process, to the amount of adherent water or trapped air bubbles going into the next chamber. In actual practice it was found that the average time in ten such transfers was six seconds and it is presumed that in the very process of the plunger going in, the adherent water from the previous chamber would be flushed out. Another alternative is to keep successive respiration chambers in a bath of water of the desired quality and transfer the plunger with the specimen to that bath and introduce them into the next chamber under water. Yet the brief exposure of the animal at the time of transfer is inevitable. Another precaution is to keep the volume of the basket or the jacket to the minimum and in any case not exceeding the volume of the reagents added, as otherwise an air pocket is bound to develop when the spare stopper is placed after the addition of the reagents.



Thus by extending the duration of the experiment progressively, the uptake of oxygen under different partial pressures of oxygen could be obtained. Furthermore, by keeping successive respiration chambers containing water in different salinities, pH or temperature *i.e.* water with various factors in different combinations, it is possible to extend the study to cover a wide range of different ambient conditions with great ease. Within any one set of conditions it is possible also to obtain replicate values by repeating the measurements as many times as one wants. These facilities are not generally available in manometric methods often employed in similar micro-estimations. If done, it would involve frequent dismantling and resetting as for instance in the Warburg apparatus.

The table below gives sample data obtained with eggs and larvae of *Tilapia mossambica* Peters at 26.5°C.

TABLE

Ave. wt of larva/egg g.	Approx. age hrs.	O_2 consump. per individdual ml/hr.
0.004	10-12	0.0016
0.009	12-16	0.0023
0.015	24	0.0025
0.017	24	0.0162
0.023	24-48	0.0019
0.025	24-48	0.0325
0.042	72	0.1247

Acknowledgment

The author expresses his thanks to Prof. C. P. Gnanamuthu, Director Madras University Zoological Research Laboratory for helpful criticism.

Reference

JOB, S. V. 1957 " The routine-active oxygen consumption of the milk fish ". *Proc. Indian Acad. Sci.*, **45** (6) B:302-313.

[Received for publication on 25th August, 1960.]

which published (only recognised abbreviations of journals, underline) Volume number (double underline) and page number. *e.g.*

Haines, R. W. 1934 The homologies of the flexor and adductor muscle of the thigh.
J. Morph., **56**, 21.

Titles of papers may be omitted altogether. Books should be listed as follows: Author's last name followed by initials, year of publication, title, publishers and place of publication. *e.g.*

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